Universidade de Aveiro Departamento de Biologia
2015

Hugo Miguel Coelho da Silva Vieira

Mercury bioaccumulation, human exposure and fish consumption recommendations regarding mercury intake

Bioacumulação de mercúrio, exposição humana e recomendações para o consumo de peixe considerando a ingestão de mercúrio

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha, realizada sob a orientação científica do Prof. Doutor Fernando Manuel Raposo Morgado, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro e co-orientação do Doutor Sizenando Nogueira de Abreu do Departamento de Biologia e Centro de Estudos do Ambiente e do Mar (CESAM), Universidade de Aveiro.

o júri

presidente

Prof. Doutor João António de Almeida Serôdio
Professor Auxiliar do Departamento de Biologia da Universidade de Aveiro

Doutor Luís Miguel Russo Vieira
Investigador Auxiliar no Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR)

Prof. Doutor Fernando Manuel Raposo Morgado
Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro

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palavras-chave

Consumo de peixe, Bioacumulação de mercúrio, Cabelo do escalpe humano, Exposição ao metilmercúrio, Isocurvas limitantes no consumo de peixe.

resumo

O Mercúrio (Hg) é classificado como um dos poluentes mais importantes devido ao seu alto grau de toxicidade, persistência e à sua capacidade de bioacumulação, especialmente no ambiente aquático. Este é libertado a partir de fontes naturais e antropogénicas, e uma vez no ambiente, o Hg inorgânico libertado pode ser convertido em Hg orgânico (metilmercúrio - MeHg) através de processos bacterianos.

O MeHg tende a bioacumular e a biomagnificar ao longo da cadeia trófica, representando um sério risco para a saúde humana. Devido a estes riscos resultantes da exposição excessiva ao Hg, agências internacionais, como a USEPA (Agência de Proteção Ambiental dos Estados Unidos) estabeleceram níveis de segurança (doses de referência (DRf)) de exposição diária, sendo a concentração de Hg presente no cabelo humano utilizada para estimar esta exposição. O peixe é uma componente importante de uma dieta saudável para a população humana e o consumo de peixe deverá ser relativamente estável nas próximas duas décadas. No entanto, o peixe também é considerado uma das principais fontes de exposição a MeHg para a população humana.

Este estudo teve como questão central a avaliação da bioacumulação de Hg em humanos face ao padrão de consumo de peixe, sendo delineados os seguintes objetivos específicos: (i) avaliar a exposição humana ao Hg face ao consumo de peixe utilizando um questionário de frequência alimentar, (ii) avaliar a exposição ao Hg através da quantificação de Hg no cabelo humano, (iii) avaliar os potenciais níveis de ingestão de Hg, através da aplicação de fórmula estabelecida pela Organização Mundial de Saúde, (iv) rever dados de consumo de peixe, valores de ingestão de Hg e conteúdo de Hg em peixes, com base em vários relatórios da Organização das Nações Unidas para a Alimentação e Agricultura, assim como, da União Europeia e (v) calcular linhas de tendência (isocurvas) descrevendo o número máximo de refeições de peixe por semana, sem exceder a DRf para o MeHg (USEPA DRf), combinando o número de refeições de peixe (por semana), a quantidade de peixe ingerido (por refeição) e a [MeHg] no peixe consumido.

Os resultados revelam que os indivíduos que indicaram consumir um maior número de refeições de peixe por semana, também apresentam em regra níveis mais altos de Hg no cabelo; no entanto, o risco de alerta para a exposição ao Hg não deve ser considerado. Os níveis reais (quantificados) e valores potenciais (extrapolados) de Hg no cabelo dos adolescentes divergiram quando aumentou o consumo de peixe, sendo menor a absorção de Hg real comparativamente ao valor esperado, dando relevo à capacidade do corpo humano induzir uma resposta de auto-protecção, sendo a absorção

de MeHg provavelmente minimizada por mecanismos de desintoxicação.

O estudo salienta que mesmo uma pequena refeição de 50g de peixe com 0,84 µg g^{-1} de MeHg por semana alcançaria do valor estabelecido para a ingestão de MeHg (DRf USEPA), apesar de ser permitido o consumo de peixe com valores de 1,0 µg g^{-1} de MeHg.

keywords

Fish consumption, Mercury bioaccumulation, Human scalp hair, Methylmercury Exposure, Limiting isocurves in fish consumption.

abstract

Mercury (Hg) is classified as a pollutant of primary importance because of its high degree of toxicity, persistence and bioaccumulative properties, especially in the aquatic environment. It is released from natural and anthropogenic sources, and once in the environment, the inorganic Hg can be converted in to organic Hg (Methylmercury – MeHg) through bacterial processes.

MeHg tends to bioaccumulate and biomagnify through the food web, representing a serious risk to human health. Due to the health risks of excessive Hg exposure, international agencies such as the USEPA (United States Environmental Protection Agency) have established safety levels (reference doses (RfD)) of daily exposure, being the Hg concentration present in human hair used to estimate MeHg exposure. Fish is an important component of a healthy diet for the human population and the fish consumption is expected to be relatively stable in the next two decades; however, fish is also considered a major source of MeHg exposure to human population.

The key question of the present study was evaluating the Hg bioaccumulation in humans based in fish consumption. Specific tasks were delineated: (i) evaluatue the human exposure to Hg via fish consumption using a food frequency questionnaire, (ii) assess Hg exposure through Hg measurement in the hair, (iii) evaluate the Hg intake levels, through the application of formulas established by the World Health Organization, (iv) review fish consumption data, Mercury Tolerable intake values and Hg content in fish, based in several reports from Food and Agriculture Organization and European Union and (v) calculation and establishment of isocurves describing the maximum number of fishmeal per week without exceeding the MeHg Rfd (USEPA RfD), by combining number of meals (per week), amount of fish ingested (by meal) and levels of MeHg in fish.

Overall data indicate that individuals consuming the highest number of fishmeals per week, also generally showed increased Hg levels in the scalp hair; however, the risk alert of the mercury exposure should not be considered. The real (quantified) and potential (extrapolated) Hg levels in human scalp of adolescents diverge as fish consumption increases, being the effective Hg uptake lower than the expected levels, emphasizing the ability of the human body to induce a self protection response, meaning that MeHg assimilation is probably minimized by detoxification mechanisms.

As a final remark, considering the intake of Hg through fish consumption as the main route exposure, the study points out that even a small meal of 50g fish with 0.84 $\mu g\ g^{-1}$ of MeHg per week would reach the USEPA RfD levels, despite the 1.0 $\mu g\ g^{-1}$ of MeHg in fish are being allowed in fish consumption.

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ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
ADI	Acceptable Daily Intake
bw	Body weight
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FFQ	Food frequency questionnaire
Hg	Mercury
IAEA	International Atomic Energy Agency
IPCS	International Programme on Chemical Safety
JECFA	Joint Expert Committee on Food Additives
MAR	Mid-Atlantic Ridge
MeHg	Methylmercury
NOAEL	No Observable Adverse Effect Level
PtEEZ	Portuguese Exclusive Economic Zone
PTWI	Provisional tolerable weekly intake
RfD	Reference dose
THg	Total mercury
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

Chapter I:

General Introduction

1. General Introduction

An increasing concern towards mercury (Hg) pollution arises with human health problems observed in various parts of the world, as a result of the Hg exposure through the consumption of seafood and marine or freshwater fish (Lin and Pehkonen 1999, Baeyens et al. 2003, Carrasco et al. 2011, You et al. 2014). The effects of Hg exposure in human health are now widely accepted (Bellinger 2014) , especially since the Hg poisoning incident which occurred in the 1950s at Minamata Bay in Japan (Ullrich et al. 2001, Rasmussen et al. 2005). Following this dramatic episode, some international agencies namely United States Environmental Protection Agency (USEPA) and World Health Organization (WHO), have established reference doses (RfD) to the mercury tolerable intake levels, aiming to prevent health risks.

Hg occupies the third position in the priority list of hazardous substances published by The US Agency for toxic substances and disease registry (ATSDR 2013), due to its persistence (Al-Majed and Preston 2000, Freire et al. 2010) and occurrence in both aquatic and terrestrial environments (Gochfeld 2003). Hg present in the environment may be originated from natural sources (Siegel and Siegel 1984, Gustin et al. 2000, Coolbaugh et al. 2002) and anthropogenic sources (Pacyna et al. 2001, Dommergue et al. 2002). In the Portuguese Mainland, one of the anthropogenic sources which contribute the most for increased Hg contamination levels are chlor-alkali plants (Mil-Homens et al. 2008), which discharge their efluents to the external environment. A known example is the Laranjo basin in Ria de Aveiro, which received a highly contaminated effluent discharged from a Hg cell chlor-alkali plant located in Estarreja industrial complex, from the 1950s until 1994 (Pereira et al. 2009). On the other hand, in the case of the volcanic Portuguese archipelagos, in particular the Azores archipelago, being a remote area from large anthropogenic sources with no significant discharge of Hg from industrial sources (Rodrigues et al. 2004), most Hg probably comes from natural sources (e.g. volcanic activity) (Afonso et al. 2007). Geochemical anomalies and hot springs are widely accepted to be natural sources of Hg (Loppi 2001).

In the environment, Hg can be found in various forms (Ullrich et al. 2001, Rasmussen et al. 2005). All forms of Hg are toxic, however, the toxicity is strongly related to the chemical properties of each form (Lindqvist and Rodhe 1985, Gochfeld 2003, Harris et al. 2003), for example, inorganic forms may be transformed in the organic form (methylated species) such as methylmercury (MeHg) through bacterial processes, like sulfate-reducing bacteria

(Compeau and Bartha 1985). When compare with other forms, MeHg is considered to be the most toxic form to aquatic organisms (Ullrich et al. 2001, Baeyens et al. 2003).

Human exposure to MeHg occurs almost exclusively through consumption of fish and shellfish since MeHg bioaccumulates in aquatic food webs (Sunderland et al. 2009). In predatory marine fish, about 90 % of the Hg exists in the methylated form (MeHg) (WHO 2008). Apart from being appointed as the main route of MeHg to humans, fish consumption is also recognized as an important component of a healthy diet (Malm et al. 1995, Mergler et al. 2007). Portugal has the highest seafood consumption per capita in Europe, corresponding to about 62 kg per year (Failler et al. 2007), occupying a top position in countries with highest seafood consumption per capita in the world (FAO 2010). In particular, the Azores archipelago is the Portuguese region with the highest consumption rate "per capita" of fishery products where each Azorean consumes about 80 kg of fish per year (Megapesca 2007).

When considering the assessment of the human population to Hg exposure through fish consumption, the human hair is a well-established matrix (Salehi and Esmaili-Sari 2010) being widely used in various studies as a biomarker for human Hg exposure (Barbosa et al. 2001, Dolbec et al. 2001, Liu et al. 2008, Salehi and Esmaili-Sari 2010, Trasande et al. 2010, Fang et al. 2012, Li et al. 2012, Shao et al. 2013), as during hair formation, Hg from the blood capillaries penetrates into the hair follicles. As hair growth is approximately 1 cm each month, Hg exposure over time is recapitulated in hair strands (WHO 1990).

2. Thesis outline and Research objectives

The general introduction and main objectives of the study are described, in the first (and present) chapter.

In the second chapter, , it was evaluated the potential risk of mercury contamination near the Mid-Atlantic Ridge relating total mercury (THg) concentrations in the human hair: “Mercury in Scalp Hair Near the Mid-Atlantic Ridge (MAR) in Relation to High Fish Consumption”.

In the third chapter, entitled “Real and Potential Mercury Accumulation in Human Scalp of Adolescents: A Case Study”, Hg accumulation and MeHg intake were assessed and extrapolated in relation to fish consumption habits in adolescents from two coastal areas.

Chapter four, entitled “Fish consumption recommendations to conform to current advice in regard to mercury intake” reviews fish consumption data, Mercury Tolerable intake values and Hg content in fish, based in several reports from Food and Agriculture Organization and European Union.

Finally, the last chapter (chapter five), integrates all the information present in the previous chapters and some final considerations were discussed as well possible future work

The major objectives of the present study were to (1) evaluate the potential risk of Hg contamination due to high levels of fish consumption in populations near the MAR, relating mercury levels in human scalp hair and fish consumption frequency in autochthone population of Terceira Island (Azores) where mercury inputs are mainly from natural sources, (2) study Hg accumulation in adolescents in relation to fish consumption in two distinct locations (Angra do Heroísmo and Murtosa), evaluating not only the effective Hg uptake registered in the human scalp hair, but also the potential levels of Hg uptake inferred from provisional tolerable weekly intake (PTWI) formulas. Actual Hg levels refer to the (effective) analytical [Hg] results measured in the scalp hair from the different fish consumption categories of volunteers. Potential Hg levels were obtained by extrapolating Hg levels in the scalp hair, derived from the application of the PTWI formula using an assumed Hg source baseline in each site and (3) assess how many meals of fish per week one can have, combining number of fish meals per week, amount of fish ingested by meal and levels of MeHg in fish without exceeding the MeHg Rfd (USEPA RfD).

To accomplish the main objectives, five specific tasks were delineated:

Evaluation of human exposure to Hg via fish consumption using a food frequency questionnaire (FFQ) (annex I) developed based on a review of the literature and other related studies.

Assessment Hg exposure through Hg measurement in the hair in a first phase in all age groups of a population in Terceira island (Azores) and in a second phase in adolescents of Terceira island (Azores) and Murtosa (Mainland). As the volunteers were minors, an informed consensus (annex II) was distributed to all volunteers who agreed to participate in the study in order to obtain authorization from the legal guardians.

Evaluation of Hg levels in the scalp hair, derived from the application of the PTWI formula calculating [Hg] in fish for the lowest class of fish consumers, and extrapolating the obtained [Hg] in fish, to the PTWI formula to higher fish consumers classes in both geographical areas, assuming [Hg] levels obtained in fish as the common Hg source, even dissimilar in the two populations.

Review fish consumption data, Mercury Tolerable intake values and Hg content in fish, based in several reports from Food and Agriculture Organization and European Union.

Calculation and establishment of isocurves describing the maximum number of fishmeal per week without exceeding the MeHg Rfd (USEPA RfD), by combining number of meals (per week), amount of fish ingested (by meal) and levels of MeHg in fish.

This study have already been published in three papers:

- Vieira, H. C., F. Morgado, A. M. V. M. Soares, and S. N. Abreu. 2013. Mercury in Scalp Hair Near the Mid-Atlantic Ridge (MAR) in Relation to High Fish Consumption. Biological Trace Element Research 156:29-35. DOI: 10.1007/s12011-013-9849-7

Link: http://dx.doi.org/10.1007/s12011-013-9849-7

- Vieira, H. C., F. Morgado, A. M. V. M. Soares, and S. N. Abreu. 2014. Real and Potential Mercury Accumulation in Human Scalp of Adolescents: A Case Study. Biological Trace Element Research 163:19-27. DOI: 10.1007/s12011-014-0159-5

Link: http://dx.doi.org/10.1007/s12011-014-0159-5

- Vieira, H. C., F. Morgado, A. M. V. M. Soares, and S. N. Abreu. 2015. Fish consumption recommendations to conform to current advice in regard to mercury intake. Environmental Science and Pollution Research:1-8. DOI: 10.1007/s11356-015-4635-z

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Chapter II:

Mercury in Scalp Hair Near the Mid-Atlantic Ridge (MAR) in Relation to High Fish Consumption

Mercury in scalp hair near the Mid Atlantic Ridge (MAR) in relation to high fish consumption

Vieira, H.C.[1], Morgado, F.[2], Soares, A.M.V.M.[2], Abreu, S. N.[2]

[1] Department of Biology; University of Aveiro, 3810-193 Aveiro, Portugal

[2] Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

1. Abstract

The aim of this study was to evaluate the potential risk of mercury contamination near the Mid Atlantic Ridge (MAR) relating total mercury (THg) concentrations in the human scalp hair (n=110) and high fish consumption levels.

THg was quantified in human scalp hair and volunteers were questioned about age, gender, smoking habits, being subsequently grouped in categories based on the individual average intake of fish meals per week.

THg concentrations ([THg]) in hair samples ranged from 0.05 to 2.24 µg g^{-1} and significant differences were found according to age (p<0.05) and also among volunteers presenting different fish consumption rates (p<0.001); being the highest [THg] observed on the adult population and also on volunteers that indicated consuming five or more meals of fish per week .

Results indicate a pattern of increased mercury accumulation with increasing fish consumption. Despite mercury availability and a potential mercury intake of up to 7 times the WHO provisional tolerable weekly intake of mercury value, in consequence of high fish consumption, mercury concentrations in scalp hair are comparatively low regarding recommended levels by WHO.

Keywords: Azores, High fish consumption, Mercury bioaccumulation, Mid Atlantic Ridge, Scalp hair

2. Introduction

Mercury (Hg) is recognized for its toxicity and ability of accumulation (Agah et al. 2007), making it one of the most hazardous elements, in the marine environment (Al-Majed and Preston 2000, Palma et al. 2009).

Mercury has the capacity to bioaccumulate in marine organisms and to biomagnify along the food chain (Renzoni et al. 1998). The bioaccumulation is the balance of uptake and elimination processes, being the rates of elimination slower than rates of uptake (Afonso et al. 2007, Magalhães et al. 2007); potentially leading to serious problem in human health (Harmelin-Vivien et al. 2009). Many studies have indicated fish consumption as the main pathway for Hg exposure in humans (Holsbeek et al. 1996, Fang et al. 2012), mostly pointing anthropogenic sources.

In environments exposed to active hydrothermal fields such along the Mid Atlantic Ridge (MAR) few studies have linked mercury accumulation with high consumption of fish. MAR is a vast underwater mountain (for over 12000 km) from Iceland to the Bouver triple junction in the south Atlantic (Dover 1995, Desbruyères et al. 2001, Kádár et al. 2005). Nine active hydrothermal vents fields are known between Ecuador and the Azores archipelago; being areas where the hydrothermal fluids, enriched in trace metals such as Fe, Co, Cu, Cd, Mn, Pb, Zn and Hg (Rousse et al. 1998, Kádár et al. 2005), blend with the seawater (mixing zone) making the hydrothermal activity an important source for the global cycle of metals in the ocean (Rousse et al. 1998). Some of these trace metals, even when available at low concentrations have been identified as toxic for living organisms (Kádár et al. 2005, Kádár et al. 2006). Carr et al. (1974) found high concentrations of mercury (0.08 – 0.4 $\mu g\ L^{-1}$) on the seawater samples near the bottom of MAR when compared with (0.002 – 0.04 $\mu g\ L^{-1}$) on samples of seawater collected away from the MAR. Later studies have correlated levels of metals found in soft tissues (especially in digestive gland and byssus) of mussel (Bathymodiolus azoricus) and the levels of metals found on water from the surrounding environment, Kádár et al. (2006). Other studies, such as Martins et al. (2006) assumed that hydrothermal vents are an additional source of mercury to deep-sea fish species that have been associated with the hydrothermal environment.

Some authors have reported the bioavailability of metals at deep sea vents depending not only on the fluid composition but also on physic-chemical factors such as temperature and pressure (Charlou et al. 2000, Kádár et al. 2005).

Azores archipelago (Fig.1), presents hydrothermal activity inside the Portuguese Exclusive Economic Zone (PtEEZ), providing natural nonpoint sources of mercury (Afonso et al. 2007) that is released into the marine environment as the result of natural processes such as volcanic eruptions and hydrothermal vents (Palma et al. 2009). Three major hydrothermal vents of the Mid Atlantic Ridge (MAR) are located near the Azores (Desbruyères et al. 2001), Menez Gwen (-850m, 37º 50`N, 31º 31`W) (Charlou et al. 2000), Lucky Strike (-1700m, 37º 18`N, 32º 16`W) (Groisillier et al. 2006) and Rainbow (-2300m, 36º 13`N, 33º 54`W) (Douville et al. 2002). Menez Gwen and Lucky Strike are inside the PtEEZ while the Rainbow is slightly farther away from the limits of the PtEEZ (Southward 1998).

Scalp hair has been used as bioindicator for metals accumulation, including as a bioindicator of mercury exposure for humans populations (Dolbec et al. 2001). There are several reasons to used scalp hair as bioindicator for mercury exposure: (1) it is easy to collect in a non-invasive manner (2) it captures temporal exposure history as Hg is incorporated into growing hair (3) it does not require special facilities for transport or storage (Cizdziel and Gerstenberger 2004). In the last two decades, several authors evaluating mercury and fish consumption (Table 1) have found total mercury (THg) levels in scalp hair ranging from 0.44 up to 11.45 μg g^{-1}.

Table 1 Levels of total Hg (μg g^{-1}) in human hair from diverse sites

Location	Mean	Range	Remark	References
Bangladesh	0.44	0.02-0.95	Fish consumption	Holsbeek et al. (1996)
Italy	0.64	0.22-3.40	Fish consumption	Díez et al. (2008)
Sweden	1.7	0.1-18.5	Fish consumption	Johnsson et al. (2004)
Sori Beach, Kenya	2.09	0.73-5.6	Fishing village	Harada et al. (2001)
Iran	3.52	0.44-53.56	Fish consumption	Salehi and Esmaili-Sari (2010)
Kuwait	4.18	–	Fisherman	Al-Majed and Preston (2000)
Colombia	4.91	–	Fishing community	Olivero et al. (2002)
Cambodia	7.3	0.54-190	Fish consumption	Agusa et al. (2005)
Malaysia	11.45	0.01-21	Fish consumption	Hajeb et al. (2008)

The United States Environmental Protection Agency (USEPA) reference of dose (RfD) of THg in hair is 1 μg g^{-1} (USEPA 1997), whereas the WHO normal level in hair is 2 μg g^{-1} (WHO 1990), while the No Observable Adverse Effect Level (NOAEL) for adults of WHO is 50 μg g^{-1}.

Azores major industries are scarce, which implies absence of significant anthropogenic mercury discharges to the environment.

Several authors have reported Hg levels in fish species that are commonly consumed in the Azores, namely *Phycis phycis*, *Trachurus picturatus*, *Helicolenus dactylopterus*, *Prionace glauca*, *Xiphias gladius*, *Aphanopus carbo* and *Mora moro*, in some cases (eg. Branco et al. (2007) and Magalhães et al. (2007)) exceeding the values for safe human consumption 0.5 μg g^{-1} (or even 1.0 μg g^{-1} in *Mora moro* (Magalhães et al. 2007) and

Aphanopus carbo (Afonso et al. (2007)) defined by European Community Regulation 78/2005 (EU 2005).

Fishery statistics for the fish consumption per capita in the Azores archipelago, show that each Azorean consumes about 80 kg of fish per year being the region with the highest consumption of fishery products (http://www.lotacor.pt/noticia.php?noticia_id=397). According to the high consumption levels would the population be at risk of mercury poisoning? Even mercury levels below the reference levels of 0.5 µg Hg g^{-1} (EU 2001, 2005) in eatable fish would lead to a potential mercury uptake up to 40 mg of mercury per year! Largely exceeding almost 7 times WHO Provisional tolerable weekly intake (PTWI) of 1.6 µg Hg g^{-1} (by body weight). Should a risk alert of the mercury exposure be considered?

The aim of this study was to evaluate the potential risk of mercury contamination due to high levels of fish consumption in populations near the MAR, relating mercury levels in human scalp hair and fish consumption frequency in autochthones population of Terceira Island (Azores) where mercury inputs are mainly from natural sources.

3. Materials and Methods

3.1. Sampling site

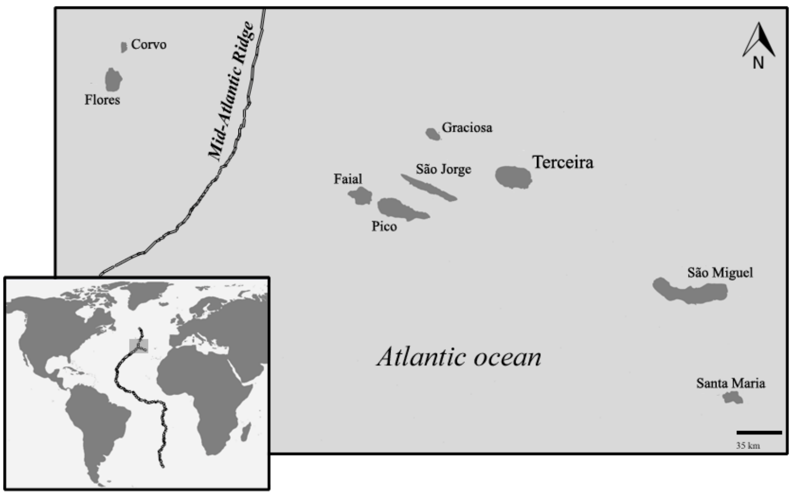

Figure 1 Map of study area (Terceira Island, Azores) enhancing the proximity of the island to the MAR

The Azores archipelago consists of nine islands and is located in the North Atlantic Ocean (Fig. 1). The Archipelago extends along a south east-north-west strip near the triple junction of Eurasian, African and North American plates. Therefore, the archipelago has a complex tectonic setting, where seismic–volcanic phenomena are common, being responsible for natural inputs of Hg to the aquatic environment.

The hair samples were collected from volunteers on Terceira island (38 º 37'N - 27 º 02'W and 38 º 48'N - 27 º 23'W) during the summer of 2011. The island (402.2 square kilometers) is crossed by the Terceira Rift, a geological structure associated with the triple junction. In spite of the geographical localization of Terceira island near the MAR, the surface fine sediments in the coastal area show relatively low levels of mercury (~0.1μg g^{-1}) (unpublished data).

3.2. Hair sampling

Scalp hair samples, were obtained randomly from 110 inhabitants (29 males and 81 females) of Terceira Island, who have been fully informed about the purpose of the study through a descriptive document.

The volunteers (from 3 to 91 years old, with a mean of 31.4 years) were classified into four groups according to the respective age (INE, 2011): children (0-14 years old), adolescents (15-24 years old), adults (25-64 years old) and seniors (>65 years old). The hair samples were obtained by a single cutting from the occipital region using clean stainless steel scissors. The samples were then kept in clean microtubes of 2 ml and identified appropriately. In the laboratory, the hair samples were cut to lengths of about 2 cm segments and washed according to the standard procedure recommended by the International Atomic Energy Agency (IAEA): wash in acetone, three times in water and once more in acetone (Cortes Toro et al. 1993). The samples were after dried overnight in an oven at 40 °C.

During scalp hair sampling, each individual was asked to complete a questionnaire detailing age, gender, body weight, height, smoking habits and frequency of fish consumption (number of meals per week).

3.3. Mercury quantification

Mercury determination was performed by atomic absorption spectrometry after thermal decomposition of the sample using the Advanced Mercury Analyser (AMA-254, LECO).

This technique of quantification is based in a pyrolysis process of the sample using a combustion tube heated at 750 ºC under an oxygen atmosphere. Volatilized mercury Hg(0) is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry (AAS) (Costley et al. 2000).

Analytical quality of the procedure was controlled using reference material TORT-2 (Lobster Hepatopancreas Reference Material for Trace Metals, National Research Council of Canada) containing 0.27±0.06 μg g^{-1} of THg and DORM-3 (Fish Protein Certified Reference Material for Trace Metals, National Research Council of Canada) containing 0.382±0.06 μg g^{-1}. Obtained data and reference values are not statistically different, with a recovery percentage of 90% and 88%, respectively.

3.4. Statistical analysis

Outliers were removed following an observation of THg boxplot and data normality was tested through Kolmogorov-Smirnov test. Data did not follow a normal distribution and the normality was established after log-transformation; therefore the parametric statistical one-way ANOVA were used to compare mercury concentration with various socioeconomic variables and Tukey test were used to determine significant differences in fish consumption groups and age groups.

Spearman correlation was used to test the correlation between THg levels and fish consumption. Statistical analysis was assessed using SPSS (version 15.0). Statistical significance was defined as $p<0.05$.

4. Results and Discussion

4.1. Mercury in scalp hair, age, gender and smoking habits

Figure 2 displays [THg] from low to high concentration of mercury in scalp hair (μg g^{-1}) considering all sampled volunteers.

Distributing THg concentration levels in scalp hair according to an increasing sequence (Fig. 2), the results shows a linear increase of THg concentration to about 1.2 μg g^{-1}, embracing elements from all age groups and consumption frequencies, illustrating a normal reference dose as suggested by USEPA. After that concentration (1.2 μg g^{-1}), the levels presented in the increasing gradient are mostly from volunteers declaring the highest fish consumption frequencies. Considering THg values above the normal mercury levels by WHO, no volunteers belonging to the children group, neither to the senior group where found. This fact might be explained by the age factor in the first group and alteration in food diet in the seniors group.

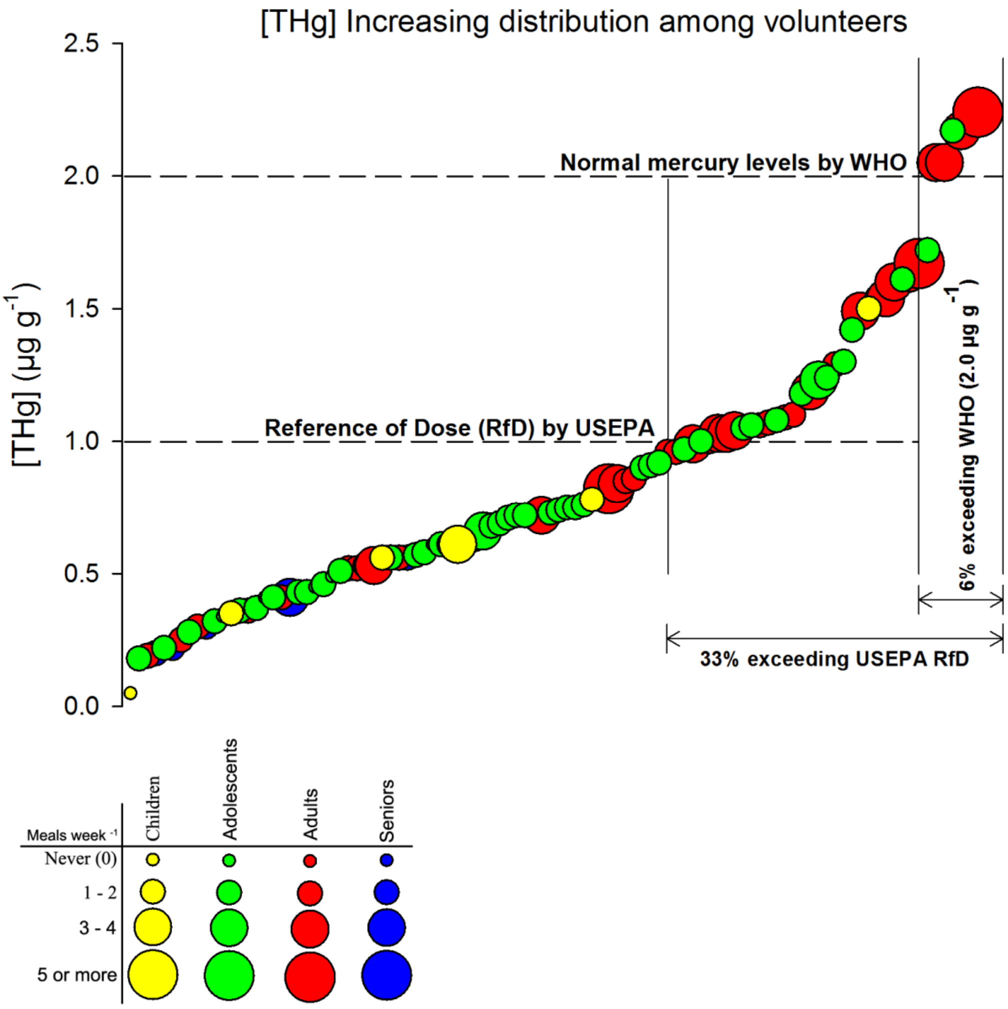

Figure 2 Graphic representation of mercury concentration [THg] (increasing gradient) enhancing age groups and meal per week frequency distribution from low to high concentration of mercury (µg g^{-1}) in scalp hair

Considering the overall average THg in the Terceira Island (0.86±0.05 µg g^{-1}), the [THg] in scalp hair are considered relatively low for a fish consuming population, regarding USEPA RfD (USEPA 1997), and also according to the WHO, normal THg level in hair (WHO 1990) (Fig. 2). In spite of that, about 33% of the sampled group in Terceira Island exceed the RfD indicated by USEPA RfD (1 µg g^{-1}), whereas 5.9% of the volunteers exceed the normal level of WHO (2 µg g^{-1}).

Considering [THg] evaluation in scalp hair among age groups (children, adolescents, adults and seniors), figure 3 illustrates a gradual increasing rate from children up to adults (about 0.0108 µg of Hg $year^{-1}$), followed by a decreasing rate till the seniors group (about 0.0152 µg of Hg $year^{-1}$).

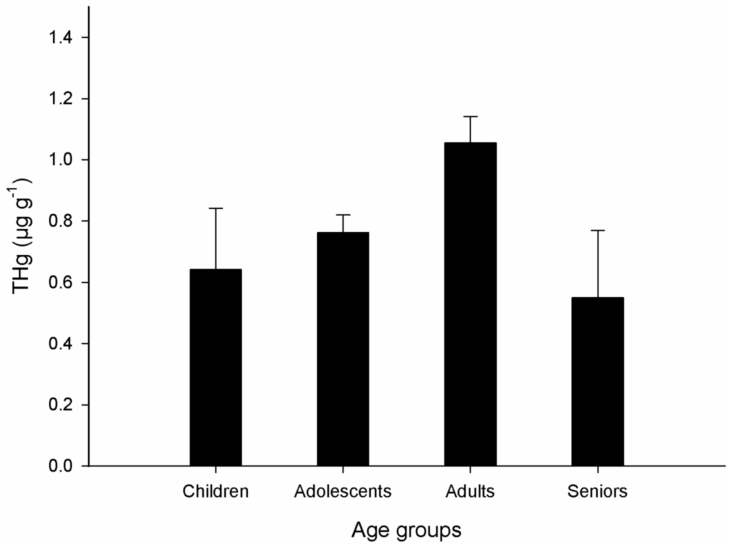

Figure 3 Mercury concentration [THg] (average and standard errors) in scalp hair in the four selected age groups (children, adolescents, adults and seniors)

Significant increase of THg levels was observed only comparing children and adults groups (p<0.05) Differences in the increment between children and adolescents groups, as well adolescents and adults groups, were not statistically significant (p>0.05). On the other hand, a significant decrease (p<0.05) of THg concentration between adults and seniors groups was observed, probably because of lower average consumption of fish meals per week in the seniors group compared to the adult group (1.83±0.31 against 2.43±0.19 meals per week). This decrease of fish meals after +65 years was also referred by Kosatsky et al (2000). The decrease of THg in scalp hair in older population was also observed in other studies such as Akira et al. (2004) and Nakagawa (1995) that found a significant decrease in THg in scalp hair after 40 years in a Japanese allergy sufferers population, and also by Al-Majed and Preston (2000) that found a small decrease of THg levels in hair after the 46 years old. Some other studies like Kim et al. (2012) and McKelvey et al. (2007) that evaluated the levels of THg in blood based on age, reported that THg levels peaked in the adult population having decreased thereafter.

Considering overall results of ANOVA, the differences in hair THg concentrations among age groups is significant (p<0.01). Comparing THg accumulation in scalp hair according to gender, data suggest higher THg levels in females (0.89±0.06 µg g^{-1}) than males (0.77±0.09 µg g^{-1}) in accordance with other results such as Agusa et al. (2005) and Hajeb et al. (2008) but also in opposition to data reported by Díez et al. (2008) and Olivero-Verbel et al. (2002), where males exhibited higher levels of THg in hair than females. Therefore, gender differences of THg levels between females and males are not significant, although they present significantly different fish consumption per week, 1.95±0.13 meals per week and 1.21±0.14 meals per week, respectively.

When taking into consideration the smoking habits, the highest mean value was found in the smokers (0.91±0.08 µg g^{-1} against 0.82±0.06 µg g^{-1} founded in non-smokers) in accordance with the results found by Kim et al. (2012) and Kosatsky et al. (2000). Although others studies such as Kowalski and Wierciński (2007) and Shao et al. (2013) have been indicating smoking habits as potential factors affecting mercury bioaccumulation in human population, our data indicates no significant differences ($p>0.05$) between smokers and not smokers.

4.2. Fish consumption and THg levels in scalp hair

Fish consumption and THg levels in scalp hair were evaluated in two scenarios: (a) considering two groups of fish consumption (fish consumers and no fish consumers) and (b) considering four groups based on fish consumption rates (meals per week).

In the first scenario, comparing two large groups: the group of volunteers that assume having consumed fish and the second group volunteers that mentioned not having consumed fish, results indicate a statistical difference ($p<0.01$) between these two groups, showing higher levels of THg in scalp hair in the fish consumers against the “no fish consumers”, respectively 0.89±0.05 µg g^{-1} and 0.39±0.08 µg g^{-1}.

The second scenario in this study, consisted in the evaluation of different frequencies of fish consumption classified into four categories: never (0), 1-2 meals per week, 3-4 meals per week and 5 or more meals per week in which 5.9% of the participants never ate fish, 73.5% ate fish 1-2 meals per week, 17.6% ate fish 3-4 meals per week and 3% ate fish more than 4 times per week and the THg values founded for each category ranged between 0.05-0.61 µg g^{-1}, 0.18-2.22 µg g^{-1}, 0.41-2.17 µg g^{-1}, 0.82-2.24 µg g^{-1}, respectively.

The levels of THg concentration indicate a significant increase from people that said not having eaten fish (never) to the people that admitted eating fish consuming five or more meals of fish per week ($p<0.001$). This significant increase was also observed among all categories except when comparing group of 3-4 meals per week and 5 or more meals per week, even though the average THg in scalp hair have increased from group 3-4 to the group 5 or more meals per week. Similar results of THg increments with number of fish meals were also reported by Al-Majed and Preston (2000), Díez et al (2008) and Shao et al. (2013) but presenting higher levels in the scalp hair.

Therefore, evaluating THg in scalp hair and fish consumption frequency, data indicates a statistical positive correlation ($p<0.005$), not only comparing fish consumers vs no fish consumers (r_s=0.267, p<0.05) but mainly comparing THg and weekly fish intakes (r_s=0.415, p<0.001) enhancing fish consumption as the potential via of mercury uptake, as also observed by other authors reporting low THg concentrations such as Díez et al. (2008) and Holsbeek et al. (1996) or in studies showing higher THg concentrations in scalp hair such as Agusa et al. (2005) and Hajeb et al. (2008).

The general low values of THg found in the scalp hair of the surveyed group suggests that in spite of potential mercury availability from juvenile waters due to the proximity to the MAR (Kádár et al. 2005), the released mercury is obviously diluted in the ocean and the bioavailability of mercury to the studied population is very low compared to other studies of dietary intake (fish consumption) in countries such as Cambodia, Colombia, Iran, Malaysia, for example (Table 1), thus, not putting at risk the studied population.

5. Conclusion

Mercury concentrations in scalp hair collected from inhabitants from Terceira Island (Azores) near the MAR indicate the intake of mercury through fish consumption as the main route exposure. Individuals who have higher fish meals per week rate also have higher levels of mercury. Similar results were found concerning to the age groups. No statistic significance was found among THg levels concerning gender or smoking habits.

Overall data indicate relatively low concentration of mercury in scalp hair, despite the high fish consumption per capita. Even being exposed in some cases to mercury levels close to the maximum reference value in eatable fish (EU 2001, 2005) potentially leading to a large mercury uptake, the THg levels in scalp hair in this population, remains around the WHO normal value (2.0 µg g^{-1}), relatively low compared to the WHO NOAEL for adults (50 µg g^{-1}); therefore, a risk alert of the mercury exposure should not be considered.

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Chapter III: Real and Potential Mercury Accumulation in Human Scalp of Adolescents: A Case Study

Real and Potential Mercury accumulation in human scalp of adolescents: a case study

Vieira, H.C.[1], Morgado, F.[2], Soares, A.M.V.M.[2], Abreu, S. N.[2]

[1] *Department of Biology; University of Aveiro, 3810-193 Aveiro, Portugal*

[2] Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

1. Abstract

Mercury (Hg) concentration in human hair is used to estimate methylmercury (MeHg) exposure and establish a Reference Dose (RfD) for MeHg intake. In this study, Hg accumulation and MeHg intake were evaluated in relation to fish consumption habits in adolescents from two coastal areas: Angra do Heroísmo (Azores archipelago) and Murtosa (Portuguese mainland).

Results showed that Hg concentration and MeHg intake increased with increasing fish consumption. In spite of that, Hg concentrations remained relatively low when compared with World Health Organization (WHO) NOAEL; therefore, risk for mercury exposure should not be considered. Adolescents revealed a similar range of Hg concentrations (0.03–2.60 µg g^{-1}) in scalp hair, apart from being exposed to natural or anthropogenic Hg source (Azores and Mainland, respectively). Nevertheless, Mainland volunteers generally exhibited higher values of Hg accumulation, being approximately 50% of the results above 1 µg g^{-1}.

Hg concentrations increased in both adolescent groups according to the weekly rate of fish meals, however not linearly in the highest fish consumption rates. In fact, considering the adolescents' group having over one fish meal per week, the Hg bioaccumulation pattern found in the respective scalp hair suggests the ability of the human body to induce a self protection response, probably mitigating Hg levels in the blood when experiencing increasing Hg exposure due to fish uptake. Actual and potential mercury levels in human scalp of adolescents probably diverge as fish consumption increases, being the effective Hg uptake lower than the expected, reducing risk to human health.

Keywords: Adolescent fish consumers, human scalp hair, mercury accumulation, Methylmercury intake.

2. Introduction

Mercury (Hg) is a ubiquitous and persistent trace metal in the environment, being considered one of the most hazardous elements, especially in the marine ecosystems (Al-Majed and Preston 2000, Palma et al. 2009, Freire et al. 2010). Hg is released into the environment from natural processes (eg. volcanic activity) and also from anthropogenic sources (eg. industrial processes) (Agusa et al. 2005, Freire et al. 2010). Intense geotectonic activity, including volcanic eruptions, geothermal vents, and degassing from the earth`s crust, has been described as an important natural source of Hg (Amaral et al. 2006, Amaral et al. 2008, Palma et al. 2009). The main cause of Hg exposure for human population is through consumption of seafood, fish and other marine animals (Al-Majed and Preston 2000, Díez et al. 2008). Hg uptake by marine organisms is a cumulative process resulting from bioaccumulation and biomagnification through the food webs. Biomagnification of Hg along the food chain ultimately targets humans, representing a serious problem to human health (Dolbec et al. 2001, Agusa et al. 2005).

Due to the health risks of excessive Hg exposure, Food and Agriculture Organization (FAO)/ World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) established a Reference dose RfD named “provisional tolerable weekly intake” (PTWI) for MeHg of 1.6 µg kg bw^{-1} week^{-1} (JECFA 2004). United States Environmental Protection Agency (USEPA) presented the lowest value for the intake of MeHg (RfD), established in 0.1 µg kg bw^{-1} day^{-1} (USEPA 1997b). PTWI and RfD correspond to a hair Hg concentration of 2.2 and 1.0 µg g^{-1}, respectively. WHO, through analysis of neurotoxicological data, considered the Hg concentration of 50 µg g^{-1} in human hair as a "no observed adversary effect level" (NOAEL) value for MeHg (WHO 1990).

Scalp hair has been used in several studies as a bioindicator of Hg exposure for human populations. Hair grows approximately 1cm each month; during hair formation, Hg, which is present in circulating blood, is incorporated into the hair follicles, where it becomes stable providing the accumulation pattern and the history of exposure (Dolbec et al. 2001, Agusa et al. 2007, Díez et al. 2008, Freire et al. 2010). This technique is advantageous as scalp hair is easy to collect in a non-invasive manner; it records chronological exposure, and it does not require special transport or storage facilities (Bencko 1995, Cizdziel and Gerstenberger 2004). Hg concentration in scalp hair is used to assess blood Hg concentrations at the moment of hair growth (USEPA 1997a) and the exposure level to methylmercury (MeHg) can also be estimated from hair Hg levels, since approximately 80% of hair Hg is MeHg (McDowell et al. 2004). A conversion factor of 250:1 (mg kg^{-1} / µg

L^{-1}) is used to convert hair Hg concentration to blood Hg concentrations (USEPA 1997b, Hightower and Moore 2003).

The aim of this work was to study Hg accumulation in adolescents in relation to fish consumption in two distinct locations (Angra do Heroísmo and Murtosa), evaluating not only the effective Hg uptake registered in the human scalp hair, but also the potential levels of Hg uptake inferred from PTWIs formulas. Volunteers were classified in different categories regarding declared fish consumption per week rates. Actual Hg levels refer to the (effective) analytical [Hg] results measured in the scalp hair from the different fish consumption categories of volunteers. Potential Hg levels were obtained by extrapolating Hg levels in the scalp hair, derived from the application of the PTWI formula using an assumed Hg source baseline in each site.

3. Materials and Methods

3.1. Sampling sites

Figure 4 Map of study area (Terceira Island, Azores and Murtosa, Mainland)

The survey was conducted in two sampling sites, one located in the Azores and other in the Portuguese Mainland (Fig. 4). Sampling sites were selected based on similar Hg levels in their surrounding coastal waters despite different mercury source, geographical localization and socioeconomic characteristics. Angra do Heroísmo is a small city located in Terceira Island on the Azores archipelago, where industrial activity is scarce; and fishery and agriculture are the main economic activities. Murtosa is located in the Portuguese mainland, and characterized mainly by industrial activities with a significant number of potential focuses of environmental pollutants including one chlor-alkali plant.

In spite of showing confined hot spots of mercury contamination inside a coastal lagoon (Abreu et al. 2000), Aveiro open sea coastal waters have relatively low mercury concentration (<0.3µg g^{-1} in surface fine sediments) (Pereira et al. 2009).

3.2. Hair sampling and analytical procedure

Scalp hair samples were obtained randomly from 157 young individuals aged 14-18 years of two different high schools: 84 inhabitants (46 females and 41 males) from Angra do Heroísmo (Azores) and 73 inhabitants (41 females and 32 males) from Murtosa (Mainland). In a first moment, volunteers were invited to participate in the study after a detailed explanation of the main objectives. Afterwards , as the volunteers are minors, an informed consensus was distributed to all volunteers who agreed to participate in the study in order to obtain authorization from the legal guardians. The legal guardians have been fully informed about the purpose of the study through a descriptive document and invited to complete a questionnaire about the volunteer, detailing age, gender, body weight, and frequency of fish consumption.

Hair samples with approximately 30 mg, were obtained by a single cutting from the occipital region using clean stainless steel scissors. Samples were then kept in clean microtubes of 2 ml and appropriately identified. At the laboratory, the hair samples were cut in segments of about 1 cm length and washed according to the standard procedure recommended by the International Atomic Energy Agency: wash in acetone, three times in Milli-Q water, and once more in acetone (Cortes Toro et al. 1993). Afterwards, samples were dried overnight in an oven at 35 °C.

3.3. Mercury Quantification

Hg quantification was performed by atomic absorption spectrometry with the Advanced Mercury Analyzer (AMA-254, made by ALTEC and distributed by LECO). Hg quantification does not requires a previous digestion of the sample; the technique is based in a pyrolysis process of the sample using a combustion tube heated at 750 °C under an oxygen atmosphere. Volatilized mercury is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry (Costley et al. 2000).

Analytical quality of the procedure was checked using the reference material TORT-2 (Lobster Hepatopancreas Reference Material for Trace Metals, National Research Council of Canada). Obtained data (0.31±0.01 μg g^{-1} of Hg) and reference 0.27±0.06 μg g^{-1} of Hg) values were not statistically different with a recovery percentage of 110%.

3.4. Calculation of exposure to MeHg

In humans, Hg concentration in blood can be related to the average MeHg daily ingestion. The concentration of Hg in hair is assumed to be 250 times the concentration in blood (USEPA 1997b).

To estimate the ingestion of MeHg (μg kg bw^{-1} day^{-1}) from a hair Hg concentration, two steps have been taken into account: a) conversion of the Hg concentration in the hair (μg g^{-1}) to that in the blood (μg L^{-1}) was done according the following equation (USEPA 1997b):

$$\frac{Hg\ hair\ (\mu g\ g^{-1})}{250} \times 1000$$

and b) conversion of the Hg blood concentration into MeHg intake can be estimated from hair Hg concentration using the following formula (USEPA 1997b):

$$d = \frac{C\ \times b\ \times V}{A\ \times f\ \times bw}$$

Where d = dose (μg MeHg kg body weight^{-1} day^{-1})

C = mercury concentration in blood (μg L^{-1})

b = elimination rate constant (0.014 per day^{-1})

V = blood volume (5 liters)

A = fraction of the dose absorbed (0.95)

f = the absorbed fraction distributed to the blood (0.05)

bw = body weight (60 kg)

and to extrapolate the MeHg intake the following formula c) was used (WHO 2008):

$$MeHg\ intake = \frac{Amount\ of\ fish\ ingested(g\ week^{-1}\ ) \times [MeHg]\ in\ fish\ ingested\ (\mu g\ g^{-1})}{Kilogram\ body\ weight\ (Kg\ bw)}$$

Through the MeHg intake obtained with the formula b), the amount of fish consumed per week and body weight, the concentration of MeHg in fish was calculated following the equation d)

$$[MeHg] in\ fish\ (ppm) = \frac{MeHg\ intake\ (\mu g\ MeHg\ kg\ body\ weight\ day)}{Amount\ of\ fish\ ingested\ (g\ week\ )} \times 60\ (Kg\ bw)$$

3.5. Statistical analysis

Outliers were removed following an observation of Hg concentration boxplot and data normality was examined through Shapiro-Wilk test. Since data was not normally distributed non-parametric statistical methods, Wilcoxon on Rank sum test and Kruskal–Wallis test, were used to compare the mean Hg concentration among the categories (local, age, gender and fish consumption). Statistical analyses were realized using SPSS (version 15.0). Statistical significance was defined as $p<0.05$.

4. Results and Discussion

Considering the USEPA RfD 1 μg g^{-1} of Hg in hair (USEPA 1997b), about a quarter of the volunteers from the Azores and half of the sampled population in the Mainland exceeded this safety standard (Fig. 5).

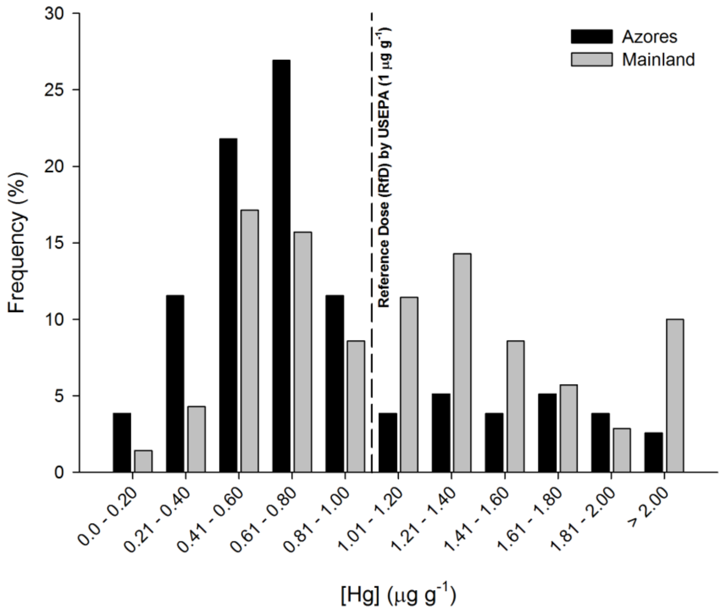

Figure 5 Distribution of [Hg] in scalp hair obtained in both sampling sites (Azores and Mainland)

The average Hg hair concentration in the Azores was 0.82±0.06 µg g^{-1} and ranged between 0.03 and 2.13 µg g^{-1}. The average Hg hair concentration in the Mainland was 1.13±0.07 µg g^{-1} varying from 0.03 µg g^{-1} to 2.60 µg g^{-1}. These results suggest higher Hg levels in the Mainland when compared to data from the Azores. The average Hg concentrations in hair scalp showed significant differences (p<0.05) between sampling sites. These differences may be related with the fish intake, as there was a significant difference (p<0.05) when comparing the meals of fish per week in the Mainland (3.31±0.22 meals $week^{-1}$) and the fish meals per week in the Azores (2.08±0.18 meals $week^{-1}$).

Regarding gender, mean Hg concentrations presented values in accordance with previous studies, in both sampling sites (Babi et al. 2000, Olivero et al. 2002). Females had higher Hg concentration levels (0.87±0.07 µg g^{-1} in Azores and 1.19±0.10 µg g^{-1} in the Mainland) in scalp hair when compared to males (0.77±0.09 µg g^{-1} in Azores and 1.03±0.10 µg g^{-1} in the Mainland). However, these gender differences were not statistically significant (p>0.05).

Gender differences in terms of Hg levels might be mainly ruled by dietary intake. Volunteers from both Azores and Mainland exhibited the same pattern of fish intake in which females showed a higher consumption of fish meals per week (2.09±0.24 and 3.49±0.31 meals $week^{-1}$, respectively) than the males (1.69±0.27 and 3.07±0.31 meals $week^{-1}$).

Hg accumulation in the scalp hair was also evaluated separately for both sampling sites (Azores and Mainland) by classifying the fish consumption of the sampled populations into four categories: never (0 meals per week), 1-2 meals per week, 3-4 meals per week and 5 or more meals per week (Fig. 6).

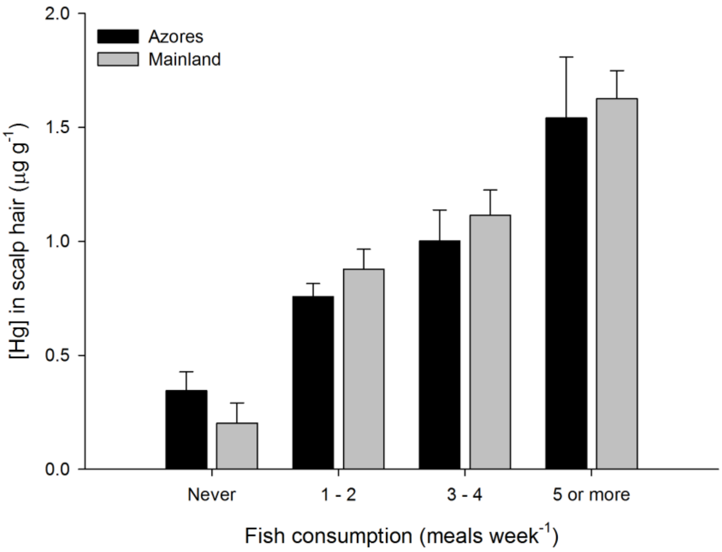

Figure 6 Average [Hg] in scalp hair in volunteers evaluated separately in both sampling sites according to fish consumption (meals per week)

The evaluation of different rates of fish consumption classified into four categories revealed that 6.4% of the Azores' volunteers and 4.3% of the Mainland volunteers assumed that they never eat fish. On the other hand, the category of 1-2 meals per week is the most representative in the Azorean volunteers, where 66.7% of these individuals assumed to have a maximum of two meals of fish per week against the 31.4% verified in the Mainland. In contrast, regarding the category of 3-4 meals per week, Mainland was the most representative, where 40% of the individuals assumed to eat fish 3 or 4 times per week in opposition to the 21.8% found in the Azores. In the last category, 5.1% in the Azores and 24.3% in the Mainland assumed to have 5 or more meals of fish per week. The Hg concentration found for each category ranged between 0.03-0.49 (never), 0.13-2.12 µg g^{-1} (1-2 meal per week), 0.28-2.07 µg g^{-1} (3-4 meals per week) and 0.95-2.00 µg g^{-1} (≥5 meals per week) in the Azores while in the Mainland it ranged between 0.03-0.32 µg g^{-1} (never), 0.27-1.77 µg g^{-1}, (1-2 meal per week) 0.41-2.47 µg g^{-1} (3-4 meals per week) and 0.93-2.60 µg g^{-1} (≥5 meals per week).

The results found in both sampling sites (Azores and the Mainland) were in accordance with Al-Majed and Preston (2000), Díez et al (2008), Vieira et al (2013) and Shao et al. (2013) which indicated an increment in Hg concentration with the increase of fish meals rate. The levels of Hg concentration indicated a significant increase in the case of the individuals who claimed to never eat fish in relation to those who admitted to consume fish 3-4 meals per week and five or more meals per week (p<0.05).

Although Mainland presented higher values in the categories of 1-2, 3-4 and ≥5 meals per week (Fig. 6), no significant differences (p>0.05) were observed when comparing categories and sampling sites.

4.1. MeHg exposure estimation using mercury concentration in scalp hair

MeHg exposure has been estimated using scalp hair as bioindicator assuming that it can assess blood Hg concentrations at the moment of hair growth (USEPA 1997a). For instance, in the assessment of the relationship between mercury concentration in maternal blood and maternal intake dose, the USEPA used a one-compartment model (Borum et al. 2001, Rice 2004), which is no more a significant simplification of the pharmacokinetics of MeHg in the maternal body and maternal–fetal unit (Rice 2004). Pharmacokinetic models have been developed for MeHg with physiological basis (Swartout and Rice 2000), however, accurate rate constants are not available for humans for the necessary compartments, including maternal blood ↔ fetal blood ↔ fetus (Rice 2004). Although these constants are not available, Ginsberg and Toal (2000), maintain that the one-compartment model predicts a half-life of elimination from maternal blood reasonably well and thus is useful for estimating the half-life in maternal blood.

Stern and Smith (2003), verify the contribution of variability in the ratio of cord:maternal blood Hg, in the toxicokinetics of the one compartment model, evaluating the ratio distributions for Hg and MeHg by means of a meta-analysis of data from previous publications and in addition, Stern (2005) conducted a probabilistic estimate of maternal RfD for men using the ratio distribution for Hg. The USEPA RfD and Stern's probabilistic RfD distribution both provide important information on risk assessment regarding prenatal MeHg (Ou et al. 2014).

After this validation and through formulas a) and b), MeHg exposure level was estimated for both sampling sites (Azores and Mainland) (table 2).

Table 2 Calculation of MeHg exposure level (µg kg bw^{-1} day^{-1}) from mercury concentrations in hair for the both sites

Site	Mean Hg in hair (µg g^{-1})	Estimated blood Hg concentration (µg L^{-1})	Exposure level (µg kg^{-1} bw day^{-1})	Exposure level (µg kg^{-1} bw week^{-1})
Azores	0.82	3.30	0.08	0.57
Mainland	1.13	4.50	0.11	0.77

To calculate the daily dose, USEPA guidelines considered the body weight of 60 kg in the denominator of the equation (USEPA 1997b). In this case study, the average body weight in Azores and Mainland was 60.5 kg and 60.8 kg, respectively; therefore, it was assumed a body weight of 60 kg for both sampling sites.

The results showed that the exposure level (µg kg^{-1} bw $week^{-1}$) in the adolescents from the Mainland is significantly higher than the average level found in Azores ($p<0.001$) (Table 2). In the Azores, the majority of the exposure levels were lower than USEPA RfD. In fact, 77.6% of the Azorean volunteers had values lower than the RfD, in contrast to the 52.2% observed in the Mainland adolescents.

Derived from the Hg concentration in scalp hair (and therefore representing the Hg concentration in the blood stream at the moment when the hair it was formed (Malm et al. 1997), calculated MeHg intake values (fig. 7) showed obviously identical trends to Hg concentration in scalp, increasing with the fish consumption similarly to other studies such as Björnberg et al. (2005) and Rubio et al. (2008). Azores volunteers that consumed more than four meals of fish per week, exhibited values above the RfD; however, in the mainland, the volunteers that had more than two meals of fish per week already exceeded this reference value.

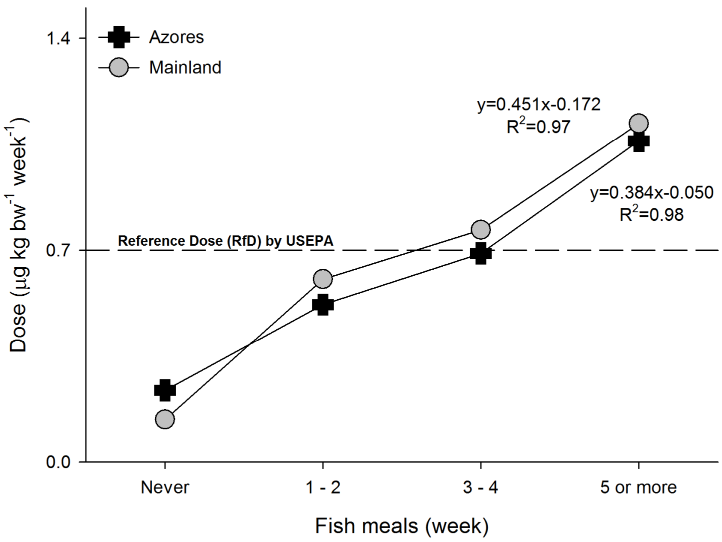

Figure 7 Average of MeHg intake (µg kg bw^{-1} $week^{-1}$) in volunteers evaluated separately in both sampling sites according to fish consumption (meals per week).

On the other hand, considering Hg concentration in the scalp hair as the result of fish consumption, the application of formula (d) would allow to estimate [MeHg] in the fish. The calculated MeHg in fish (Fig. 8) show levels of 0.16 µg g^{-1} and 0.13 µg g^{-1} (Azores and

Mainland, respectively) for the 1-2 meals of fish per week groups, but surprisingly indicates decreased levels of MeHg (<0.1 µg g^{-1}) in the higher rates of fish consumption in both sites. The amount (mass) of fish obviously increases in the higher rates of fish consumption (higher fish intake) but the corresponding Hg burden is not linearly assimilated. The estimated concentrations of MeHg in the source (ingested fish) would point a decreasing level of mercury in the fish towards higher rates of fish consumption, what it would not be acceptable assuming fish as the common source of MeHg to all fish consumption categories; having one meal of fish per week or five meals of fish per week, it would have had no effect to the Hg concentration in the fish! This way, MeHg levels in fish (considered the main Hg source) would have to be considered similar, independently of the consumer category (1-2, 3-4, and 5 or more than 5 meals per week). Thus, assuming a common level of MeHg in fish for every consumption category, the potential levels of Hg in scalp hair would have to be higher than the determined [Hg] (found in scalp hair) that our results first had indicated.

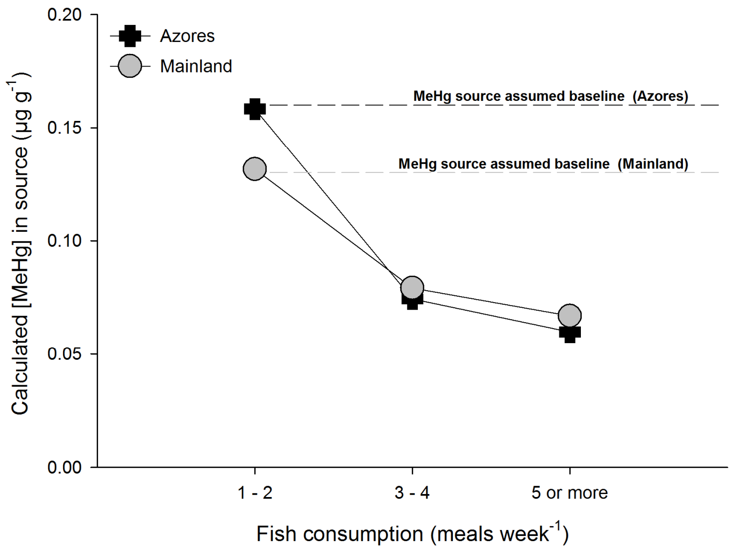

Figure 8 Estimated average of [MeHg] in source (ingested fish) versus fish consumption (meals per week)

This way, the potential intake of MeHg, meaning the levels of MeHg that would have been observed in scalp hair if the human body had not activate protective defences against MeHg assimilation, it was (back) extrapolated by (re)applying formula c) and considering the amount of fish ingested per week (170g of fish per meal) and the average MeHg levels in fish calculated for the lowest category of fish consumption (1-2 fish meals per week) in each site, Azores and Mainland, respectively 0.16 µg g^{-1} and 0.13 µg g^{-1} (assumed baseline). Indeed, considering these average values of MeHg in fish and formula c), the potential MeHg intake for the three fish consumption categories (1-2, 3-4 and 5 or more meals of fish per week) would be higher in both sites (fig. 9).

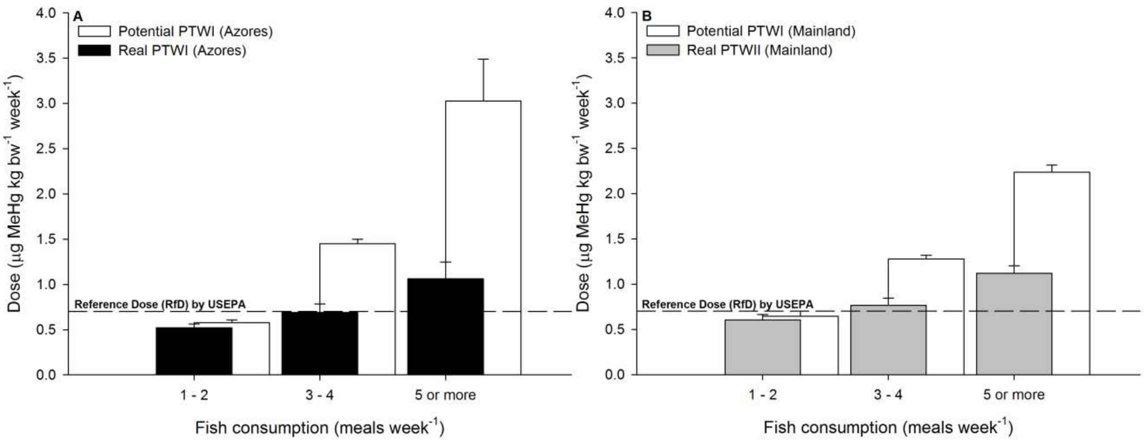

Figure 9 Real (quantified) MeHg and potential (extrapolated) MeHg intake versus fish consumption for Azores (A) and Mainland (B)

The extrapolated intake increased linearly with the fish consumption (y=1.225x-0.764; R^2=0.97 in Azores and y=0.803x-0.242, R^2=0.99 in Mainland). The differences between intakes are approximately the same when compared with the real intake for the fish consumption of 1-2 meals per week, approximately 2-fold in the volunteers that consume 2-3 fish meals per week in the both sites and approximately 3-fold and 2-fold in the category of 5 or more fish meals per week in the Azores and the Mainland, respectively.

Metals, such as Hg, are known to induce metallothioneins (Bucheli and Fent 1995, Yasutake et al. 1998) that have high cysteine content, acting similarly to metal chelators, providing heavy metal tolerance and regulating Hg distribution and retention (Yoshida et al. 1997, Klaassen and Liu 1998, Yoshida et al. 1999). Chelators of Hg (e.g., glutathione and N-acetylcysteine) have been shown to enhance the clearance of Hg from the blood perhaps by reducing metal uptake by erythrocytes (Gochfeld 1997). Thus, when Hg uptake increases (higher number of fish meals per week) it promotes the presence of metallothioneins that may reduce the presence of Hg in the erythrocytes at the hair follicle in moment of hair formation, and contributing to the deviation of the observed [MeHg] in the scalp hair, compared to the extrapolated [MeHg] when applying formula c). Micronutrients such as selenium, methionine, cysteine, and vitamin E can also protect against Hg bioactivity, likely via antioxidant responses (e.g., bcl2 gene induction in the kidney, free radical scavenging) trapping Hg and it may also aid in explaining Hg detoxification (Drasch et al. 1996), leading to a lower Hg concentration compared to the expected (extrapolated) values.

5. Conclusion

The main route of Hg exposure in the sampled populations is through fish consumption. Individuals that consumed the highest number of fish meals per week, also generally showed increased Hg levels in the scalp hair. Mainland volunteers exhibited higher fish consumption (1.6 fold) and higher levels of Hg in the scalp hair (1.3 fold) when compared to the Azorean volunteers; however, the risk alert of the mercury exposure should not be considered.

Hg concentration in human hair is assumed to reflect MeHg intake, allowing to evaluate the risk associated to the MeHg intake for the human population. This study indicates that real (quantified) and potential mercury levels in human scalp of adolescents diverge as fish consumption increases, being the effective Hg uptake lower than the expected. These results emphasize the ability of the human body to induce a self protection response, minimizing MeHg assimilation probably by detoxification mechanisms, thereby mitigating Hg levels in the blood when experiencing increasing Hg exposure.

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Chapter IV:
Fish consumption recommendations to conform to current advice in regard to mercury intake

Fish consumption recommendations to conform to current advice in regard to mercury intake

Vieira, H.C.[1], Morgado, F.[2], Soares, A.M.V.M.[2], Abreu, S. N.[2]

[1] *Department of Biology; University of Aveiro, 3810-193 Aveiro, Portugal*

[2] *Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal*

1. Abstract

This article reviews fish consumption data, Mercury Tolerable intake values and Mercury (Hg) content in fish, based in several reports from Food and Agriculture Organization and European Union. The study assumptions are valid based on the current established USEPA reference dose (RfD).

Combining number of meals (per week), amount of fish ingested (by meal) and levels of MeHg in fish, this study calculates and presents isocurves indicating the maximum number of fishmeal per week without exceeding the methylmercury (MeHg) reference dose (USEPA RfD). RfD are assumed to be the “exposure dose that is likely to be without deleterious effect even if continued exposure occurs over a lifetime”.

The study points out that even considering a single 50g fish meal per week, the USEPA RfD would be exceeded triggered by values above 0.84 µg g^{-1} of MeHg in fish; and this, despite of being allowed levels up to 1.0 µg g^{-1} of MeHg in fish consumption! - Have we a health risk?

Fish consumption is expected to be relatively stable, while anthropogenic mercury emissions are expected to stabilize or even to increase beyond current values. How many meals of fish per week can we have, combining number of fish meals per week, amount of fish ingested by meal and levels of MeHg in fish?

Keywords: Fish consumption, Isocurves fish consumption, Methylmercury Exposure, Mercury Tolerable intake, Reference dose

2. Introduction

Fish are beneficial to human health because of its high nutritional value. Fish are rich in protein with essential amino acids, macro elements (calcium and phosphorus), microelements (selenium and zinc), vitamins and unsaturated fatty acids. Due to its content on n-3 fatty acids, fish are considered an important source of n-3 fatty acids such as docosahexaenoic acid (22:6, n-3, DHA), eicosapentaenoic acid (20:5, n-3, EPA) (Egeland and Middaugh 1997, WHO 2003, FAO 2012). All these elements play an important role in the prevention of development of some diseases, especially regarding cardiac and circulatory disorders and they reduce mortality in patients with coronary diseases (Kris-Etherton et al. 2002).

The values of fish consumption vary according to regions and countries, due to different levels of fish availability including the accessibility of fishery resources in adjacent waters as well food traditions. The apparent average of annual per capita of fish consumption (2007-2009) around the world can vary from less than 1 kg in a country, to more than 100 kg in another (Fig. 10) (FAO 2012).

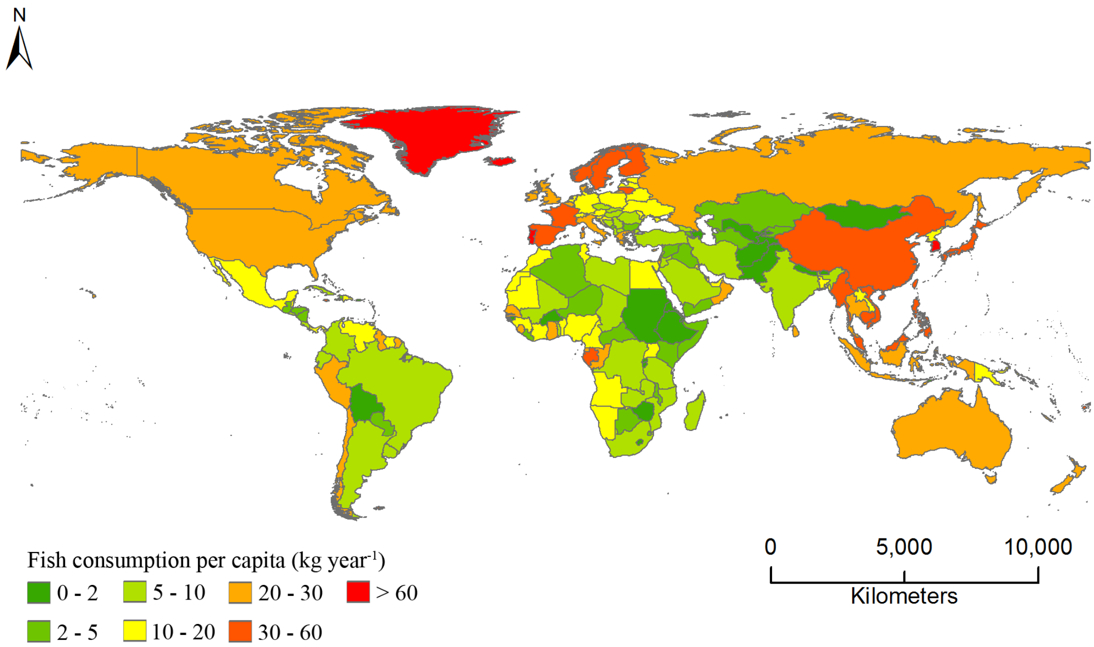

Figure 10 Fish supply per capita (average 2007-2009), adapted from FAO (2012).

Concerning the overall 126 million tonnes available for human consumption in 2009, Asia represents two-thirds of the total consumption with 85.4 million tonnes (20.7 kg per capita), of which only 42.8 million tonnes were consumed outside China (15.4 kg per capita). The lowest fish consumption was registered in Africa with 9.1 million tonnes (9.1 kg per capita), while the corresponding per capita fish consumption values for Oceania, North America, Europe, and Latin America and the Caribbean were 24.6 kg, 24.1 kg, 22.0 kg and 9.9 kg, respectively (FAO 2012).

The successive enlargement of Europe since its creation in 1957 by the Treaty of Rome, (15 Member States in 2000 (EU-15), to 21 in 2005 (EUR-21) and 28 in 2010 (EUR-28)) makes the European Union (EU) one of most important markets for aquatic products in the world, with 370 million consumers among the 15 member states and a potential market of more than 480 million with the inclusion of new member states (Failler et al. 2007). In addition, the EU is a major consumption market of seafood products in the world with 12,3 million tonnes, representing EUR 52,2 billion in 2011. EU is the first importer of seafood products, absorbing 24% of total world exchanges in value, alongside Japan and the United States of America (EUMOFA 2014).

Table 3 Fish consumption per capita (Kg year^{-1}) for all EUR-28 countries from 1989 to 2030, adapted from Failler et al. (2007).

	1989	1994	1998	2005	2010	2015	2020	2025	2030
Austria	9	12	11	11	11	12	12	12	13
Belgium-Luxembourg	21	23	22	22	22	23	23	23	24
Denmark	20	25	23	24	25	26	27	28	29
Finland	33	34	34	34	35	35	36	36	37
France	30	30	32	32	32	32	32	33	33
Germany	11	13	15	15	15	16	16	17	18
Greece	20	26	26	26	26	26	27	27	27
Ireland	22	19	21	21	21	21	21	21	20
Italy	21	22	23	24	25	26	27	28	29
Netherlands	14	16	15	15	15	15	15	16	16
Portugal	59	60	61	60	59	59	58	58	57
Spain	39	40	41	40	39	39	39	39	39
Sweden	22	27	29	28	28	27	27	27	27
United kingdom	22	20	24	24	24	25	25	25	25
EU-15 average	**23**	**24**	**25**	**26**	**26**	**26**	**26**	**27**	**27**
Cyprus	18	20	25	25	24	24	23	23	23
Czech Republic		9	9	10	10	11	11	12	13
Estonia		37	15	14	14	14	14	14	14
Hungary		4	4	5	5	5	5	6	6
Poland	15	13	11	12	13	13	14	15	16
Slovenia		6	7	7	7	8	8	8	9
EUR-6 NC average	**15**	**11**	**10**	**10**	**11**	**12**	**12**	**13**	**14**
Bulgaria		2	4	5	5	6	6	7	7
Latvia		43	37	37	37	38	38	38	39
Lithuania		21	15	17	19	21	23	25	27
Malta	23	22	29	30	31	32	33	34	36
Norway	45	47	46	46	45	45	45	45	45
Romania	9	3	3	3	4	4	4	5	5
Slovakia		7	5	6	6	7	7	8	8
EUR-7 NC average	**42**	**37**	**40**	**11**	**11**	**12**	**12**	**13**	**13**
EUR-28 avarage	**22**	**21**	**22**	**22**	**22**	**23**	**23**	**24**	**24**

The estimation of future demand has been performed through the analysis of the past patterns, based in recent trends of the commodities consumption, and also on the experts' knowledge and literature review (table 3) (Failler et al. 2007). Available data and the estimation of future fish consumption per capita (1989-2030) indicate an increasing pattern in Austria, Belgium-Luxembourg, Denmark, Finland, France, Germany, Greece, Italy, Netherlands, United Kingdom, Czech Republic, Hungary, Poland, Slovenia, Bulgaria, Latvia, Lithuania, Malta, Romania and Slovakia; while, on the other hand, a decreasing pattern was observed up to present and it has been suggested for the future in Ireland, Portugal, Spain, Sweden, Cyprus, Estonia and Norway.

All fish consumers are potentially exposed to methylmercury (MeHg). In predatory marine fish, about 90 % of the mercury (Hg) exists in the methylated form (MeHg) (WHO 2008),which is rapidly absorbed (about 95 %) through the gastrointestinal tract and assimilated penetrating easily into blood-brain and crossing placental barriers in humans and animals (JECFA 2004). After absorption, MeHg has a relatively long biological half-life in humans estimated to range from 44 to 80 days (USEPA 1997b).

Fish consumption is expected to be relatively stable, while anthropogenic Hg emissions are expected to stabilize or even to increase beyond current values. How many meals of fish per week can we have, combining number of fish meals per week, amount of fish ingested by meal and levels of MeHg in fish?

3. Fish consumption and Mercury

Apart from being recognized as an important component of a healthy diet, fish consumption has also been appointed as the main route of MeHg to humans (Malm et al. 1995, Mergler et al. 2007), leading to the establishing of reference doses to the mercury tolerable intake levels, aiming to prevent health risks from Hg exposure.

Hg has been regarded as a pollutant of primary importance since the occurrence of Hg poisoning in the 1950`s resulting in adverse effects for human health (Renzoni et al. 1998). Hg is now considered ubiquitous, persistent (Goldman and Shannon 2001, Wolkin et al. 2012) and one of the most hazardous toxicants in the environment (Morel et al. 1998, Raimundo et al. 2010).

Hg has been released to the environment from both natural and anthropogenic sources (Hansen and Danscher 1997, Voegborlo et al. 2010). Volcanic emissions, geothermal releases and biomass burning are the main natural sources while mining, chloroalkali production and fossil fuels combustion are the most described anthropogenic sources (Renzoni et al. 1998, Pirrone et al. 2010). Every year, large amounts of Hg are released to the atmosphere, being about 5207 tonnes from natural sources and 2320 tonnes from anthropogenic sources (Pirrone et al. 2010). Atmospheric Hg is eventually deposited back onto land and water. Hg is released predominantly in a inorganic form (Hansen and Danscher 1997, Baeyens et al. 2003) but it can be converted in MeHg by aerobic and anaerobic bacteria (Cizdziel and Gerstenberger 2004, Raimundo et al. 2010).

In the environment, MeHg tends to bioaccumulate and biomagnify through the food web, reaching concentrations many times higher than the levels in the surrounding water (Miklavčič et al. 2013) and ultimately representing a serious risk to human health. The term “risk” is defined by The International Programme on Chemical Safety (IPCS) as being the probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent (IPCS 2004).

This way, despite being fish a significant source of protein (Burger 2005, Mergler et al. 2007), dietary fish intake is also a major MeHg source to the general fish consumers (USEPA 1997a, Horvat et al. 1999). Therefore, populations with a traditionally high dietary intake of fish are the most exposed to MeHg bioaccumulation and may consequently experience its deleterious effects in human health (Burger 2005, Pirrone et al. 2010).

Considering MeHg intake and associated risk, some definitions such as Acceptable Daily Intake (ADI), Reference Dose (RfD) and also Provisional Tolerable Weekly Intake (PTWI) have been implemented. IPCS refers ADI as “a estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed daily over their lifetimes without appreciable health risk” and RfD as “an estimate of the daily exposure dose that is likely to be without deleterious effect even if continued exposure occurs over a lifetime” (IPCS 2004). PTWI is defined by Joint FAO/WHO Expert Committee on Food Additives (JECFA), as “the value that represents permissible human weekly exposure for food contaminants such as heavy metals with cumulative properties” (WHO 1987).

The majority of the countries in the world are members of FAO (Fig. 11) and in these countries (except in the Japan where it is 0.3 μg g^{-1}) the maximum limit of Hg in fish for human consumption are 0.5 μg g^{-1}, but also with an exception list of species such as sharks, swordfish and tuna where the limit is 1.0 μg g^{-1}. Above these levels the fish is not recommended for consumption (EU 2005, Codex Alimentarius Commission 2011).

Also concerning human safety, USEPA recommend keeping the whole blood Hg level < 5.0 μg L^{-1} and the hair level <1.0 μg g^{-1}, corresponding to a RfD of no greater than 0.1 μg MeHg kg bw^{-1} day^{-1} (USEPA 1997a). Recently (more precisely in 2010), the JECFA reviewed the previous PTWI of 5 μg kg^{-1} body weight (bw) for Hg and it established a new PTWI for Hg down to 4 μg kg^{-1} bw which has been also considered applicable to dietary exposure to Hg from foods other than fish and shellfish (JECFA 2010). PTWI for MeHg was also subsequently revised in 2012 by European Food Safety Authority (EFSA) from 1.6 μg kg bw^{-1} $week^{-1}$ to 1.3 μg kg bw^{-1} $week^{-1}$, which is equivalent to 0.19 μg kg^{-1} day^{-1} (EFSA 2012).

Considering present fish consumption levels and the present "accepted" USEPA RfD, would be acceptable to ask: - Are we having today health risk scenarios occurring daily worldwide?

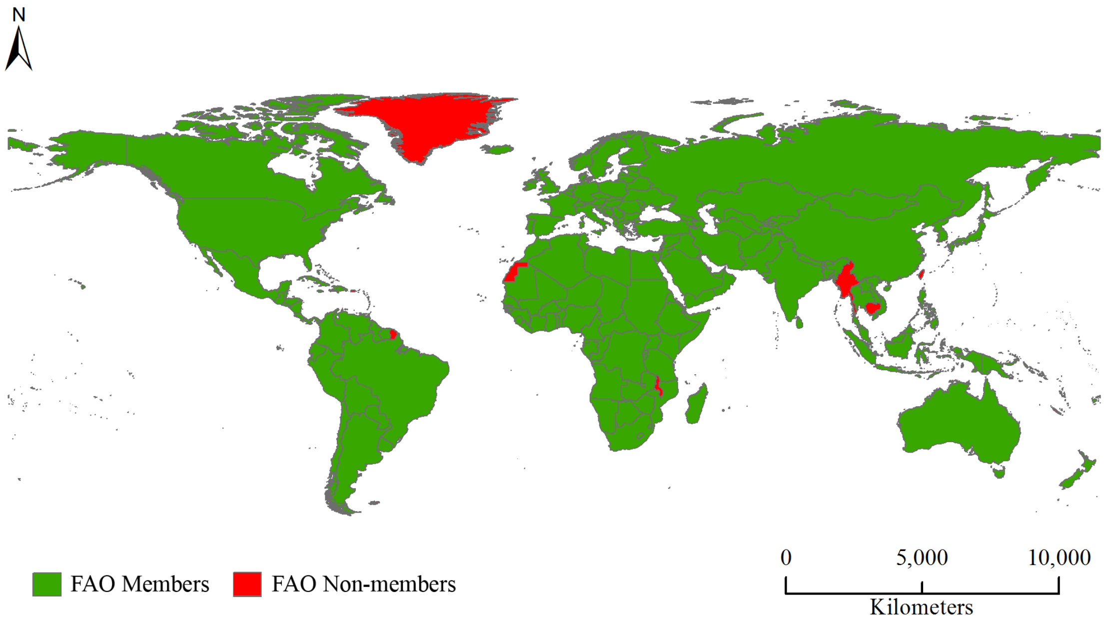

Figure 11 Distribution of FAO Members and FAO Non-members countries around the world

In order to protect public health, European Community has established maximum levels of Hg for certain contaminants in foodstuffs considering the maximum level of 0.5µg g^{-1} for most fish species, with exception for some specific species to which the maximum limit was established of 1µg g^{-1} (EU 1993, 2001). In 2005, the commission regulation (EC 78/2005) amended the previous regulation and extended the number of species covered by the "exception list" (EU 2005).

In recent years, international and national food safety agencies have recognized the need to provide useful, clear and relevant information to populations that are concerned about making the healthiest choices when considering whether or not to eat fish.

According to FAO/WHO (2011), and based on five databases from four countries: France, Japan, Norway and the USA, a new database was created presenting the average content of Hg on muscle for 77 fish species (table 4) indicating common name (and also whether it had been farmed or wild caught) for three species: salmon, rainbow trout and halibut. In the new database, the mercury content is provided as Hg instead of MeHg, and the Expert Consultation assumed 100 percent of Hg is present as MeHg, for the purposes of risk–benefit comparison.

Table 4 Concentration of Hg (µg g^{-1}) content in 77 fish species

[Hg] (µg g^{-1})	Fish (Common name)
≤0.1	Anchovy; Butterfish; Catfish; Cod, Atlantic; Cod, Pacific; Croaker, Atlantic; Flatfish; Haddock; Herring; John Dory; Mackerel; Perch, ocean and mullet; Pike; Plaice (European); Pollock; Rainbow trout (farmed); Redfish; Saithe; Salmon, Atlantic (wild); Salmon, Atlantic (farmed); Salmon, Pacific (wild); Sardines; Smelt; Sole; Sprat; Sweetfish; Tilapia; Wolf fish
>0.1 & ≤0.5	Anglerfish; Bass, freshwater; Bass, saltwater; Bluefish; Carp; Catshark; Croaker, Pacific; Dab; Eel; Goatfish; Grenadier; Grouper; Gurnard; Hake; Halibut, Atlantic (farmed); Halibut, Greenland; Hoki; Ling; Lingcod and scorpionfish; Mackerel, horse; Mackerel, Pacific; Mackerel, Spanish; Monkfish; Nile perch; Perch, freshwater; Pout; Sablefish; Scorpion fish; Seabass; Seabream; Skate/ray; Snapper, porgy and sheepshead; Tilefish, Atlantic; Tuna; Tuna, albacore; Tuna, skipjack; Tuna, yellowfin; Tusk; Whiting
>0.5 & ≤1.0	Alfonsino; Mackerel, king; Marlin; Orange roughy; Shark; Tuna, Atlantic bluefin; Tuna, bigeye; Tuna, Pacific bluefin
>1.0	Swordfish; Tilefish, gulf

Among these 77 fish species, 36% of species exhibit values below 0.1 µg g^{-1}, 49.4% ranging from 0.1 to 0.5 µg g^{-1}, while 11.7% presented values between 0.5 and 1 µg g^{-1} and only 2.6% exhibit values above 1 µg g^{-1}.

4. Fish consumption and Methylmercury exposure

International agencies such as the USEPA and JECFA have established safety levels of daily exposure based on the epidemiological studies in New Zealand, Seychelles and the Faeroe (Burger et al. 2001, Rice 2004, Maycock and Benford 2007). USEPA fixed the guideline values for maximum exposure limits (RfD) of 0.1 µg MeHg kg bw^{-1} day^{-1} (USEPA 1997a) and JECFA established PTWI equivalent to 0.19 µg kg^{-1} day^{-1} for MeHg (EFSA 2012). These guidelines were established applying a 10-fold Uncertainty Factor (UF) intended to account for the variation among healthy human population prolonged exposed to contaminated fish consumption (USEPA 1997b). Latterly, Grandjean and Budtz-Jørgensen (2007), suggested the adjustment to a maximum exposure limit of 0.05 µg MeHg kg bw^{-1} day^{-1} (50% USEPA RfD) based on studies of developmental neurotoxicity at exposure levels close to 50% below USEPA RfD (Jedrychowski et al. 2007, Lederman et al. 2008, Suzuki et al. 2010).

The level of exposure to MeHg depends on i) type and amount of ingested fish per unit time (such as day or week); ii) MeHg concentrations in fish; and, iii) body weight of the fish consumers. Thus, MeHg intake (µg MeHg kg bw^{-1} $week^{-1}$ for individuals or populations) can be calculated by the following the formula e) (WHO 2008):

$$MeHg\ intake = \frac{Amount\ of\ fish\ ingested\ (g\ week^{-1}) \times [Hg] in\ fish\ ingested (\mu g\ g^{-1})}{Kilogram\ body\ weigth\ (Kg\ bw)}$$

Therefore, assuming a human body weight of 60kg (based on USEPA guidelines, (USEPA 1997c) and considering USEPA RfD of 0.1 µg MeHg kg bw^{-1} day^{-1} or 0.05 µg MeHg kg bw^{-1} day^{-1} (as maximum exposure levels), the maximum values of fish meals per week can be calculated using fish weight (50, 100, 150, 170, 200 or 250 g of fish per meal) and [MeHg] in ingested fish (ranging from 0.01 to 1.0 µg MeHg g^{-1}, accepted levels in fish consumption):

$$Fish\ meal\ week = \frac{(RfD \times bw) \times 7 days}{(Fish\ meal\ size\ (g) \times [MeHg] in\ fish) \div 1000}$$

The results are shown as isocurve trend lines indicating the meals of fish per week allowed, combining ingested fish weight and ingested fish contamination, being restricted for a human maximum exposure level of 0.1 µg MeHg kg bw^{-1} day^{-1} (Fig. 12a) or 0.05 µg MeHg kg bw^{-1} day^{-1} (Fig. 12b). Each isocurve represents a specific fish weight (50, 100, 150, 170, 200 or 250 g of fish per meal) under different MeHg concentrations [MeHg].

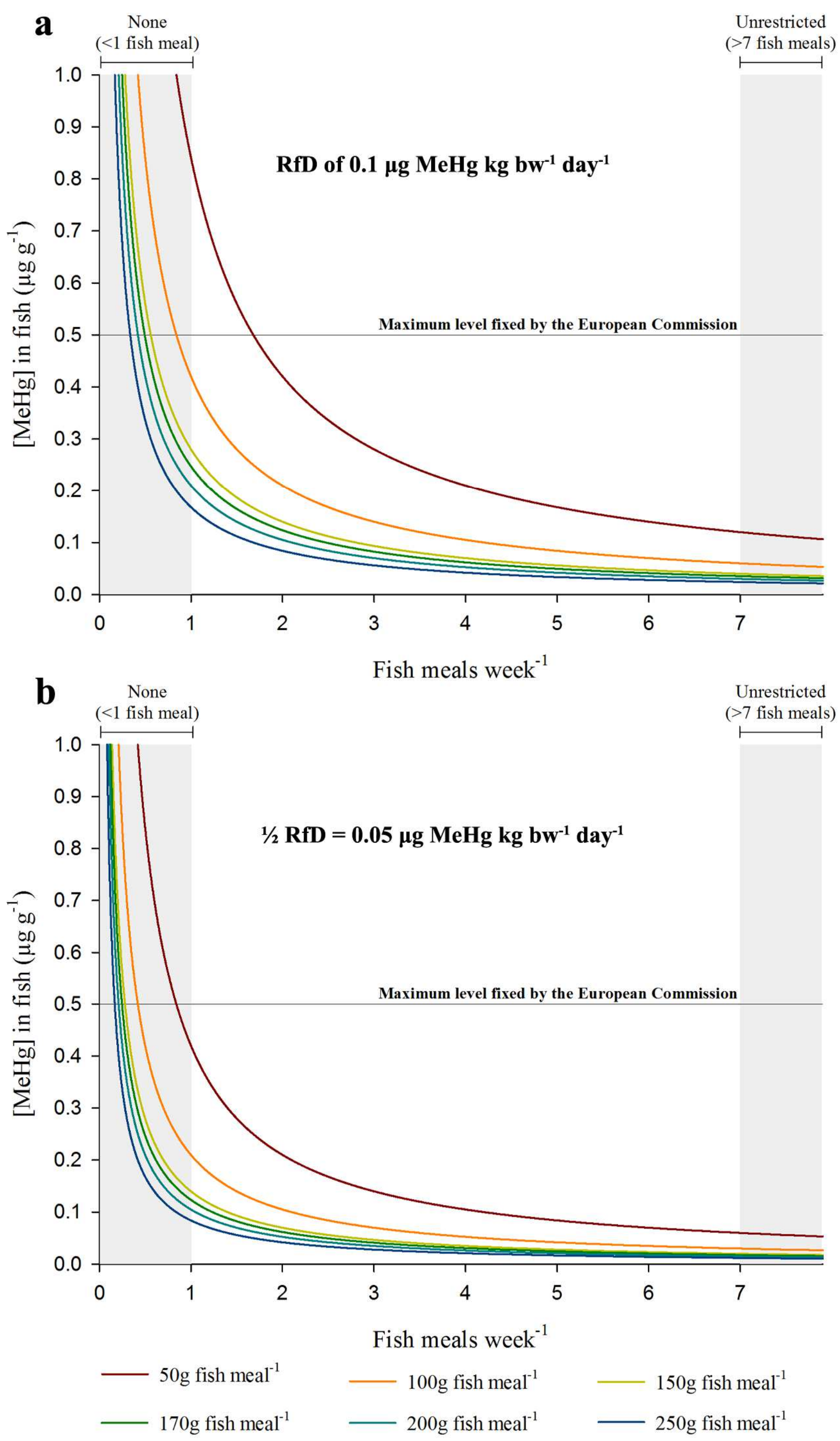

Figure 12 Fish meal week^{-1} in relation to the [MeHg] in fish (µg g^{-1}) for: a) RfD of 0.1 µg MeHg kg bw^{-1} day^{-1} and b) ½ RfD (0.05 µg MeHg kg bw^{-1} day^{-1})

The trend lines, indicating the maximum values of fish meals per week for the RfD of 0.1 µg MeHg kg bw^{-1} day^{-1} (Fig 12a), points out that regarding the fish size of 50g per meal with values above 0.84 µg g^{-1} of MeHg in the fish, it is not possible to make one meal per week without potentially exceeding the RfD, whereas that for the half of USEPA RfD, 0.05 µg MeHg kg bw^{-1} day^{-1} (Fig 12b) that value should not exceed 0.42 µg of MeHg g^{-1} of fish. As the size of fish in the meal increases, the maximum values of MeHg in fish would have to decrease, aiming a specific level of exposure. For example, to the RfD of 0.1 µg MeHg kg bw^{-1} day^{-1} (Fig 3a) and the fish size of 100, 150, 170, 200 and 250g the concentration of MeHg in fish would not have to exceed the 0.42, 0.28, 0.24, 0.21 and 0.17 µg g^{-1} respectively, following that RfD. Regarding the RfD of 0.05 µg MeHg kg bw^{-1} day^{-1} and for the fish size of 100, 150, 170, 200 and 250g, the values related to MeHg in fish would obviously have to decreased to half when compared to the values of fish meal size using USEPA RfD.

5. Discussion

Fish is an important component to a healthy diet for human population (Mahaffey et al. 2011, Nielsen et al. 2014) and fish consumption is associated with a reduced risk of coronary heart disease. About 20% of the world's population obtains at least one-fifth of its animal protein from fish intake, and some small island states depend almost exclusively on fish (WHO 2003). However, fish is also considered a major source of MeHg, a well documented neurotoxic that is linked to a loss in Intelligence Quotient (IQ), particularly when the exposure occurs in the development phase of the human being (Mahaffey et al. 2011, Bellanger et al. 2013). Therefore, it is necessary to take into account the benefits and risks associated with the consumption of fish.

The 2010 Dietary Guidelines for Americans advised Americans to consume 227 g of seafood per week to reach an average intake of 250 mg day^{-1} of the omega-3 fatty acids EPA and DHA (Nesheim and Nestle 2014) and it indicated that 4 seafood varieties should not be consumed by pregnant women: shark, tilefish, swordfish, and king mackerel (Nielsen et al. 2014). Therefore, and taking into account present review, it is questionable whether a greater number of species should be avoided, minimizing the exposure to MeHg as recommended by USEPA.

In the future, anthropogenic mercury emissions are expected to rise or remain close to current values, mainly due to the expansion of coal combustion projected to Asia (Streets et al. 2009), therefore, fish Hg levels and consequent exposure of human populations to MeHg tend to increase, since MeHg production in ocean ecosystems is driven by inorganic mercury supply available (Selin 2009, Sunderland and Selin 2013). For example Sunderland et al. (2009) suggest, based in data and modeling a relatively rapid near term, an increase in Hg concentrations in the North Pacific Ocean over the next several decades if contemporary atmospheric deposition rates are maintained. Such increases could have serious implications for resulting contaminant burdens in fish if methylated Hg species production mimics total Hg concentration trends. Therefore, sooner or later, as fish is an important component of human diet, elevated MeHg exposure will require changes in fish consumption considering both risks and benefits.

In this article, a survey data for fish consumption per capita and Hg content in fish it was performed based in several reports of Food and Agriculture Organization (FAO) and European Union (EU) (Failler et al. 2007, FAO/WHO 2011, FAO 2012, EUMOFA 2014). Subsequently, the maximum values of fish meals per week were evaluated based on formula b), in order to establish the maximum weekly consumption of fish without exceeding Hg USEPA RfD. Therefore, the resulting isocurves describes the maximum number of fishmeal per week without exceeding the MeHg reference dose (USEPA RfD), combining number of meals (per week), amount of fish ingested (by meal) and levels of MeHg in fish and these isolines are easy to calculate from person to person taking into account both personal references and fish consumption habits.

These assumptions are valid based on the current established USEPA RfD, which have been supported by scientifically well conducted and well designed field studies (Rice et al. 2000) involving human exposure to MeHg concerning fish-eating populations (Burger et al. 2003; Dourson et al. 2001; Rice 2004). However, despite being considered accurate, RfDs have also not been pointed as precise estimates of doses below a toxicity threshold (Dourson et al. 2001; USEPA 2002).

6. Conclusions

Fish consumption and associated risks of MeHg intake have always been a worldwide concern. Some guidance levels such as ADI, PTWI and RfD, have been considered in order to prevent deleterious effect even if continued exposure occurs over a lifetime. Despite that, fish consuming is today, and also it will be in the future, an essential protein source to human population.

Several scenarios regarding fish consumption were considered regarding guidance levels published in the literature. The study combines number of meals, amount of fish ingested and having as food source fish (showing Hg values accepted by current international legislation). The results were described by isocurves where each isocurve represents a scenario (number of meals, fish weight, and MeHg in fish) aiming not to exceed maximum exposure dose. Encompassing all data, it is concluded that even a single meal (per week) of 50g fish with 0.84 µg g^{-1} of MeHg would reach the USEPA RfD maximum levels (Fig. 3a), despite the 1.0 µg g^{-1} of MeHg in fish are being allowed in fish consumption. In addition, taking into account both, the USEPA RfD and a meal of fish having the maximum limit of 0.5 µg g^{-1} Hg in fish, only a consumption of 50g fish per week would be safe without exceeding USEPA RfD.

Finally, and to ultimately protect fish consumers health, more dietary and specific strategies would be required, such as simplified forms of communication, in order to promote and improve the consumer understanding of the risk-benefit of fish consumption, and most of all, longer-term efforts to reduce uptake contaminants levels through pollution monitoring.

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Chapter V:

Final Considerations

1. Final considerations

Since the occurrence of the Hg poisoning episode in the Minamata Bay in Japan, numerous studies have shown that contaminated fish consumption is the major source leading to human exposure to MeHg. mainly from the larger predatory fish which contain much higher levels of MeHg than non-predatory fish. Portugal has one of the world's largest fish consumption rate, however the existing studies considering this thematic have been rare.

The studies presented in this thesis represent a contribution to the knowledge of Hg bioaccumulation in humans through fish consumption in Portugal and also debate fish consumption and MeHg exposure in a global scale.

To improve our understanding of Hg bioaccumulation in humans through fish consumption, a sequential work based in three steps was performed: i) a initial study based in the evaluation of the potential risk of Hg contamination near the MAR relating THg concentrations in the human scalp hair and high fish consumption levels; ii) a second study aiming to determine Hg accumulation and MeHg intake in relation to fish consumption habits in adolescents from two coastal areas with different sources of Hg and the evaluation not only the effective Hg uptake registered in the human scalp hair, but also the potential levels of Hg uptake inferred from PTWIs formulas; and finally, iii) a review on the available fish consumption data, Mercury Tolerable intake values and Hg content in fish, based in several reports from Food and Agriculture Organization and European Union, as well as combining the number of meals (per week), amount of fish ingested (by meal) and levels of MeHg in fish. This last study also involved the calculation of isocurves indicating the maximum number of fishmeal per week, enhancing limits without exceeding the methylmercury (MeHg) reference dose.

Individuals that consumed the highest number of fishmeals per week, also generally showed increased Hg levels in the scalp hair, however, the risk alert of the mercury exposure should not be considered. The real (quantified) and potential Hg levels in human scalp of adolescents diverge as fish consumption increases, being the effective Hg uptake lower than the expected, emphasizing the ability of the human body to induce a self protection response, as MeHg assimilation is probably minimized by detoxification mechanisms.

As a final remark, and considering the intake of Hg through fish consumption as the main route exposure, the study points out that even a small meal of 50g fish with 0.84 $\mu g\ g^{-1}$ of

MeHg per week would reach the USEPA RfD maximum levels, despite the 1.0 µg g^{-1} of MeHg in fish are being allowed in fish consumption.

2. Future work

This work presents pertinent questions concerning human exposure and mercury bioaccumulation on a global scale and also represents one step forward in the assessment of Hg exposure of Portuguese Population through fish consumption. The knowledge acquired with this thesis brings new questions that may be addressed in future research.

The possible future work in this field includes improving knowledge through studies of: a) the occurrence and distribution of Hg along the Portuguese coast and archipelagos, since, despite an existing knowledge of the occurrence and distribution of Hg in the mainland, this information regarding the archipelagos is scarce, b) mercury bioaccumulation quantification at different trophic levels in the marine food chain would be helpful to a better understanding of how these compounds move from the lower levels to the upper levels of the marine food chain in the PtEEZ, c) collect and analyze the Hg content in the fish species available in the Portuguese fish market, in order to obtain more realistic data on mercury exposure of the Portuguese population and d) investigate the enzymatic response (detoxification) of the studied organisms when exposed to mercury.

On a global scale, the review study also questions assumptions accepted worldwide related to human exposure and current tolerance reference levels. Have we a human health risk problem or should these reference levels be reviewed?

ANNEXES

Food frequency questionnaire

QUESTIONÁRIO BIOACUMULAÇÃO MERCÚRIO EM ADOLESCENTES

Amostra Nº ______

ANGRA DO HEROÍSMO ☐ MURTOSA ☐

1. INFORMAÇÕES GERAIS (ASSINALAR COM X)

Sexo: MASCULINO ☐ FEMININO ☐

IDADE ☐ PROFISSÃO ☐

PESO ☐ Kg FREGUESIA ☐

Altura ☐ m

2. FUMADOR

SIM ☐ NÃO ☐

2.1. SE SIM, QUAL A MARCA DE TABACO (ASSINALAR COM X)

ALÉM MAR ☐ Boa Viagem ☐ Danilos ☐ L&M Vermelho ☐ MARLBORO VERMELHO ☐ SG Gigante ☐ SG Ligth ☐ Outro* ☐

*

2.2. CONSUMO DIARIO DE CIGARROS (ASSINALAR COM X)

0 a 5 ☐ 5 a 10 ☐ 10 A 15 ☐ 15 A 20 ☐ > 20

3. AMALGAMA DENTÁRIA (ASSINALAR COM X)

SIM* ☐ NÃO ☐

3.1. SE SIM

Numero de dentes ☐

3.1.1. Data da ultima colocação

0 a 5 ANOS ☐ 5 a 10 ANOS ☐ 10 A 15 ANOS ☐ 15 A 20 ANOS ☐ +20 ANOS

4. CONSUMO DE PEIXE

4.1.NUMERO DE REFEIÇÕES SEMANAIS DE PEIXE (ASSINALAR COM X)

0x☐ 1x ☐ 2x ☐ 3x ☐ 4x ☐ 5x ☐ 6x ☐ 7x ☐ 8x ☐ 9x ☐ 10x ☐ +10x ☐

4.2. Frequência e Principais espécies consumidas (Assinalar com x)

NOTA: (0 não consume; 5 consome muito)

Espécie	0	1	2	3	4	5	Espécie	0	1	2	3	4	5	Espécie	0	1	2	3	4	5
Abrótea							**Chicharro**							**Peixe-espada-branco**						
Alfonsim							**Dourada**							**Peixe-espada-preto**						
Atum fresco							**Faneca**							**Robalo**						
Bicuda							**Garoupa**							**Savel**						
Boca-negra							**Goraz**							**Solha**						
Bodião-vermelho							**Imperador**							**Taínha**						
Cântaro							**Linguado**							**Veja**						
Carapau							**Pargo**							***Outros**						
Cavala							**Peixão**													

*

4.3. Compra do Peixe (assinalar com X)

Vendedor ambulante ☐ Fresco Peixaria local ☐ Congelado ☐ Hipermercado ☐

4.4. Quantidades de Peixe consumidas semanalmente

< 200 g ☐ >200 g) ☐

4.5. Numero de refeições semanais de atum em lata (assinalar com X)

0x☐ 1x ☐ 2x ☐ 3x ☐ 4x ☐ 5x ☐ 6x ☐ 7x ☐ 8x ☐ 9x ☐ 10x ☐ +10x ☐

Marca	Azeite	Oleo	Natural
Atum Ramirez			
Atum Continente			
Atum Bom Petisco			
Atum Calvo			
Atum Vasco da Gama			
Atum Tenório			
Atum É			
Atum Santa Catarina			
Outro: ______________________________			

4.5.1. Numero de latas semanais

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐ +10 ☐

5. Consumo semanal de polvo e lula

5.1. Polvo (assinalar com X)

Raramente (0 a 2x) ☐ Ocasionalmente (4 a 6x) ☐ Frequentemente (+6)☐

5.1.1. POLVO

LOCAL ☐ IMPORTADO ☐

5.2. QUANTIDADES DE POLVO CONSUMIDAS MENSALMENTE

< 200 g ☐ >200 g) ☐

5.3. LULAS (ASSINALAR COM X)

RARAMENTE (0 A 2X) ☐ OCASIONALMENTE (4 A 6X) ☐ FREQUENTEMENTE (+6X)☐

5.3.1. LULAS

LOCAL ☐ IMPORTADO ☐

5.4. QUANTIDADES DE LULAS CONSUMIDAS MENSALMENTE

< 200 g ☐ >200 g) ☐

HUGO VIEIRA
DEPARTAMENTO DE BIOLOGIA
UNIVERSIDADE DE AVEIRO
3810-193 AVEIRO
PORTUGAL
HUGOVIEIRA@UA.PT

Informed consensus

Consentimento Informado

Hugo Vieira, biólogo licenciado pela Universidade de Aveiro, pretende realizar um trabalho de investigação científica no âmbito de mestrado, visando a "Acumulação de mercúrio em adolescentes: um caso de estudo comparando populações portuguesas expostas a fontes naturais e antropogénicas" mediante a determinação de mercúrio em cabelos (cerca de 0,01g serão suficientes e não deixa qualquer marca visível), assim sendo venho por este meio solicitar a colaboração de V.ª Excelência.

As amostras de cabelo serão obtidas através de um corte único da região occipital (vulgarmente conhecido por "nuca") usando uma tesoura de aço inox limpa e desinfetada. Durante a amostragem de cabelo da zona de escalpe, cada voluntario será convidado a preencher um questionário detalhando idade, género, peso corporal, altura, hábitos de fumo, frequência de consumo de peixe (número de refeições por semana).e espécies de peixe mais consumidas.

Neste estudo, serão levantadas algumas questões:

a) Haverá diferença na acumulação de mercúrio em relação à fonte de mercúrio (natural ou antropogénica)?
b) Será que os níveis de mercúrio nos cabelos incrementam com o aumento da idade?
c) Número de refeições semanais de peixe poderá influenciar a bioacumulação de mercúrio?

____
Hugo Vieira
Departamento de Biologia
Universidade de Aveiro
3810-193 Aveiro
PORTUGAL

hugovieira@ua.pt

✂..

Autorização

Eu,____, Encarregado de Educação do aluno ____, declaro que **AUTORIZO** o meu educando a participar na amostragem para o "Estudo da acumulação de mercúrio em adolescentes", acima referido, sendo seguro que se trata de um processo confidencial e que os dados pessoais não serão publicados sem o meu consentimento.

__________________, ___ de _____________ de 2013

Assinatura do Encarregado de Educação, ____

Contato (tlm / telf / e-mail):__________________________________